

Hematopathology

Morphology, Immunophenotype,
Cytogenetics and Molecular
Approaches



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30 Corporate Drive, Suite 400, Burlington, MA 01803, USA
84 Theobald's Road, London WC1X 8RR, UK
Radarweg 29, PO Box 211, 1000 AE Amsterdam, The Netherlands

First edition 2008

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British Library Cataloguing-in-Publication Data

A catalogue record for this book is available from the British Library

Library of Congress Cataloging-in-Publication Data

A catalog record for this book is available from the Library of Congress

ISBN: 978-0-12-370607-2

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Typeset by Charon Tec Ltd., A Macmillan Company.
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To my Grandchildren Arya and Shayan
Faramarz Naeim

To my Father
P. Nagesh Rao

To my Father
Wayne W. Grody

Preface

The main purpose of this book is to provide the reader a relatively comprehensive and concise source of information on the topic of hematopathology. The authors have selected a multidisciplinary approach by correlating morphology with biochemistry, immunophenotyping, cytogenetics and fluorescence *in-situ* hybridization, molecular studies, and clinical aspects. This book offers important information to practicing physicians and those in pathology and hematology training, which will help them to better understand the nature of the hematologic disorders and improve their diagnostic skills along the way. It also functions as a valuable referral book for researchers who work in hematology-related areas.

The book consists of 24 chapters. The first 6 chapters are devoted to normal structure and function of hematopoietic tissues, principles of immunophenotyping, cytogenetics and molecular genetics, an overview of abnormal bone marrow morphology, and reactive lymphadenopathies. Chapters 7 through 24 deal with various types of clonal and non-clonal hematopoietic disorders. These disorders are classified according to the currently published classification by the World Health Organization (WHO).

In planning this book, the authors considered that while there are some excellent

hematopathology texts and atlases available, no single volume combines the two in a way that reflects the thought processes a pathologist goes through in evaluating a real-world case. The explosion of new molecular, cytogenetic, and proteomic techniques applicable to pathology has not rendered histologic examination obsolete but rather offers powerful ancillary information to facilitate differential diagnosis, predict prognostic behavior, and help in the selection of targeted molecular therapies. With this in mind, we have constructed most chapters along the general format for each disease category to encompass etiology and pathogenesis, pathologic features (morphology, immunophenotype, cytogenetics, and molecular studies), clinical aspects, and differential diagnosis. To facilitate a better understanding of the text material and the grasp of the information, numerous tables and images are provided throughout the book. Images represent and illustrate the characteristic features of morphology, immunophenotype, cytogenetics, and molecular studies in the majority of the disorders discussed in the book.

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Acknowledgements

We are grateful for the support and encouragement of Farhad Moatamed, M.D., Department of Pathology and Laboratory Medicine, Department of Veterans Affairs, Greater Los Angeles Healthcare System. He generously facilitated our work and offered valuable advice throughout the process of this production.

The authors are thankful to Drs. Sophie Song and Tom Howard for their contributions in writing Chapters 17 and 18, and Chapter 24, respectively.

Preparation and completion of this book would not have been possible without the technical and laboratory assistance of Myrna Fisher

and Cecille Repinsky in flow cytometry, Diana Tanaka-Mukai and Eva Archuleta in hematology, and Ivanna Klisak and Audry Teng in cytogenetics. The authors also acknowledge all the Residents, Fellows, and Staff at VA Greater Los Angeles Healthcare System and UCLA for providing the opportunity to teach, learn, and exchange ideas that are reflected in this book.

We would also like to thank Mara E. Conner, the Publishing Editor, and Megan Wickline, the Developmental Editor of Academic Press, for their assistance in the production of this book.

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Structure and Function of Hematopoietic Tissues

BONE MARROW STRUCTURE AND FUNCTION

Bone marrow is a mesenchymal-derived complex structure consisting of hematopoietic precursors and a complex microenvironment that facilitates the maintenance of hematopoietic stem cells (HSCs) and supports the differentiation and maturation of the progenitors. All differentiated hematopoietic cells including lymphocytes, erythrocytes, granulocytes, macrophages, and platelets are derived from HSCs.

In the early embryonic life, HSCs first appear in yolk sac and mesodermal tissue of the aorta-gonad-mesonephros region [1–3]. These stem cells then migrate and colonize in a series of early hematopoietic sites including liver, thymus, spleen, and omentum [1–3]. They eventually reside in bone marrow as their permanent home, where they give rise to sequential generations of blood cells throughout adult life. Stem cells have highly specific homing properties, demonstrate very high self-renewal potential, and are capable of differentiation. They share morphologic features of blast cells but are distinguished by their functional properties, such as various colony-forming units (CFUs) and expression of certain differentiation-associated macromolecules. The most primitive (pluripotent) HSCs express CD34 and are negative for CD38 and HLA-DR [4–6]. These primitive cells, which include long-term repopulating stem cells, are also characterized by low level expression of c-kit receptor (CD117) and absence of lineage specific maturation markers. There is a spectrum of heterogeneity in the bone marrow stem cell pool: a continuum of cells with decreasing capacity for self-renewal and increasing potential for differentiation. This trend is also associated with

changes in immunophenotypic features. For example, the committed stem cells (short-term repopulating cells), in addition to CD34, appear to express CD38 and/or HLA-DR. The pluripotent HSCs comprise about 1 per 20,000 of bone marrow cells, and only a small fraction of them are active, whereas the remaining majority are in a “resting” phase, on call for action when it is necessary [5–7]. Based on the “clonal succession” hypothesis, a series of stem cells successively contribute to the clonal expansion to maintain a balanced hematopoiesis throughout life [8–10].

The choice of the bone marrow stem cells between self-renewal and differentiation appears to be stochastic, meaning that the commitment of a stem cell to self-renewal or to a particular pair of progeny of given differential potential is a random event and follows the probability rules of statistics [11–13]. In this random process, activation of certain complex nuclear transcription factors appears to play an important role.

Similar to the hematopoietic cells, bone marrow stromal cells are derived from pluripotent stem cells [14–16]. In other words, two separate and distinct pluripotent stem cells are simultaneously at work in bone marrow: hematopoietic and stromal. These two systems not only co-exist but closely interact with each other. Stromal cells are composed of a heterogeneous cell population including adipocytes, fibroblast-like cells, endothelial cells, and osteoblasts [17–20]. They produce a number of cytokines and a group of proteins that are involved in facilitating cell-cell interactions and presenting the cytokines and growth factors to the hematopoietic progenitor cells (Table 1.1). Stromal cells with their extracellular matrix make a mesh of fibrovascular environment to home and support the hematopoietic precursors [20–25]. The thin-walled

Faramarz Naeim

TABLE 1.1 The main adhesion molecule families.

Adhesion molecule families	Major distribution	Ligand/matrix
<i>(a) Leukocyte cell adhesion molecules (Leu CAM)</i>		
CD11a (LFA-1 α)	Leukocytes	ICAM-1
CD11b (MAC-1)	Neutrophils, monocytes	C3bi, ICAM-1
CD11c (gp150/95)	Granulocytes, monocytes	C3bi
CD18 (LFA-1 β)	Widespread	CD11a, b, c
<i>(b) Immunoglobulin superfamily</i>		
CD2 (LFA-2)	T lymphocytes	LFA-3
CD50 (ICAM-3)	Leukocytes	LFA-1
CD58 (LFA-3)	Widespread	CD2
CD54 (ICAM-1)	Widespread	LFA-1
CD102 (ICAM-2)	Endothelial cells	LFA-1
CD106 (VCAM-1)	Dendritic cells, endothelial cells	VLA-4
ICAM-4	Erythroid	AlphaVbeta3
<i>(c) Selectins</i>		
CD62E (E-selectin)	Activated endothelial cells	Sialylated Lewis-X
CD62L (L-selectin)	Leukocytes	CD34
CD62P (P-selectin)	Megakaryocytes, endothelial	Sialylated Lewis-X
<i>(d) Sialomucins</i>		
CD34	Stem cells, blasts	L-selectin
CD45	Hematopoietic cells	Heparan sulfate
CD162 (PSGL-1)	Leukocytes	Selectins
<i>(e) Very late activation antigens (VLA)</i>		
CD49a (VAL-1)	Lymphocytes, fibroblasts	Laminin
CD49b (VAL-2)	Fibroblasts, megakaryocytes	Collagen
CD49c (VAL-3)	Stromal cells	Fibronectin, laminin, collagen
CD49d (VAL-4)	Widespread	VCAM-1, fibronectin
CD49e (VAL-5)	Erythroid precursors	Fibronectin
CD49f (VAL-6)	Widespread	Laminin

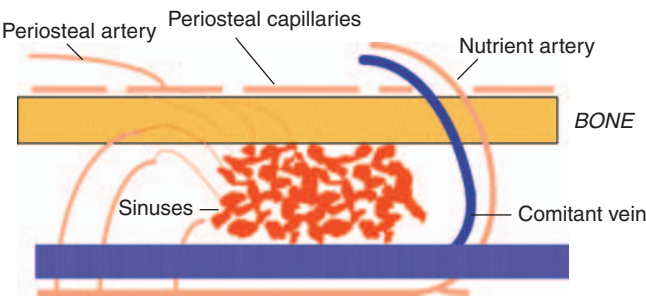


FIGURE 1.1 Schematic of microvascular circulation in the bone marrow. Adapted from De Bruyn PPH. (1981). Structural substrates of bone marrow function. *Semin Hematol* **18**, 179.

TABLE 1.2 Major components of extracellular matrix in bone marrow.

Type	Comments
Collagen (reticulin)	Consisting of various subtypes. Erythroid and myeloid precursors adhere to collagen types I and VI.
Fibronectin	Attaches to early erythroid precursors and other hematopoietic and stromal cells.
Hemonectin	Myeloid precursors adhere to laminin. Regulates leukocyte chemotaxis.
Proteoglycans	Components containing heparin sulfate, chondroitin sulfate, and hyaluronic acid. Interact with laminin and type IV collagen and play a role in cytokine presentation and cell differentiation.
Thrombospondin	Interacts with collagen, fibronectin, and CD36.

venous sinuses are the most prominent vascular spaces in the bone marrow. They consist of an inside layer of endothelial cells supported by an outer layer of fibroblast-like (parasinial, adventitial) stromal cells. They receive blood from the branches of the nutrient artery and periosteal capillary network. The nutrient artery penetrates the bony shaft, branches into the bone marrow cavity, and forms capillary–venous junctions [26, 27]. The periosteal capillary network connects with the sinuses at the bone marrow junction through the Haversian canals. The smaller venous sinuses drain into larger centrally located sinuses, which connect together to form the comitant vein. The comitant vein and the nutrient artery run through the bone marrow cavity adjacent to one another in the same vascular canal (Figure 1.1).

The HSCs reside in microenvironmental niches. These niches, which are composed of stromal cells, accessory cells (such as T lymphocytes and macrophages), components of extracellular matrix (Table 1.2), and various regulatory cytokines (Table 1.3), play an important role in the regulation of hematopoiesis and proliferation of the committed stem cells [28–30]. These niches create topographical patterns in the bone marrow [25, 31–32]. For example, granulopoiesis primarily takes place in periosteal areas where the concentration of hemonectin is higher. Recent studies suggest

TABLE 1.3 Regulatory cytokines.

Cytokine	Primary effect
GM-CSF ¹	Granulocyte and macrophage colony formation, functional enhancement of mature forms
G-CSF ²	Granulocyte colony formation, functional enhancement of granulocytes
M-CSF (CSF-1) ³	Macrophage colony formation, functional enhancement of monocytes and macrophages
Erythropoietin (EPO)	Erythropoiesis, possible enhancement of megakaryocyte proliferation
Thrombopoietin (TPO)	Megakaryocyte proliferation, platelet production
Steel factor (c-kit ligand)	Stem cell and mast cell proliferations
Interleukin (IL)-1	Promoter of hematopoiesis, inducer of other factors, B- and T-cell regulators, endogenous pyogen
IL-2	T-cell growth factor, may inhibit G/M colony formation and erythropoiesis
IL-3 (multi-CSF)	G/M colony formation, syngeneic effects on EPO, eosinophil, mast cell, and megakaryocyte colony formation
IL-4	B-cell proliferation, IgE production
IL-5	Eosinophil growth and B-cell differentiation
IL-6	B-cell differentiation, synergistic effects on IL-1
IL-7	Development of B- and T-cell precursors
IL-8	Granulocyte chemotactic factor
IL-9	Growth of mast cells and T-cells
IL-10	Inhibitor of inflammatory and immune responses
IL-11	Synergistic effects on growth of stem cells and megakaryocytes
IL-12	Promoter of Th1 and suppressor of Th2 functions
IL-13	B-cell proliferation, IgE production
IL-15	Activates T-cells, neutrophils and macrophages
IL-16	Chemotactic factor for helper T-cells
IL-17	Promotes T-cell proliferation, pro-inflammatory activities
IL-18	Activates T-cells, neutrophils, and fibroblasts
IL-19	Member of IL-10 family, transcriptional activator of IL-10
IL-20	Member of IL-10 family with epidermal function
IL-21	Improves proliferation of T- and B-cells, and enhances NK cytotoxic activities
IL-22	Member of IL-10 family; induces inflammatory responses
IL-23	Activates autoimmune responses
IL-24	Member of IL-10 family, tumor suppressor molecule
IL-25	Capable of amplifying allergic inflammation
IL-26	Member of IL-10 family; plays a role in mucosal and cutaneous immunity
TGF- β ⁴	Suppresses BFU-E, CFU-S, and HPP-CFC
Interferons	Suppress BFU-E, CFU-GEMM, and CFU-GM
TNF ⁵ - α and - β	Suppress BFU-E, CFU-GEMM, and CFU-GM
PGE ⁶ -1 and -2	Suppress GFU-GM, GFU-G, and GFU-M
Lactoferrin	Suppresses release of IL-1

¹ Granulocyte and macrophage colony-stimulating factor.² Granulocyte colony-stimulating factor.³ Macrophage colony-stimulating factor.⁴ Transforming growth factor.⁵ Tumor necrosis factor.⁶ Prostaglandin E.

that endosteal osteoblasts and their precursors play a role in the creation of stem cell niches. Osteoblasts release regulatory components that influence stem cell function, such as G-CSF, M-CSF, GM-CSF, IL-1, and IL-6, and others [14]. Erythropoiesis takes place in distinct anatomical foci referred to as “erythroblastic islands.” These islands are rich in fibronectin and consist of erythroid precursors surrounding a central macrophage [33, 34]. They are usually away from bone trabeculae and are located subjacent to the vascular structures. Megakaryocytes are mostly located in proximity of the sinuses. In this location a portion of their cytoplasm enters into the sinusoidal space to release platelets. The bone marrow niches support and regulate hematopoiesis, leading to the production of huge numbers of progenitor cells and differentiated mature blood cells (Figure 1.2).

Every day, an estimated 2.5 billion red cells, 2.5 billion platelets, and 1.0 billion granulocytes are produced per kilogram body weight in normal conditions.

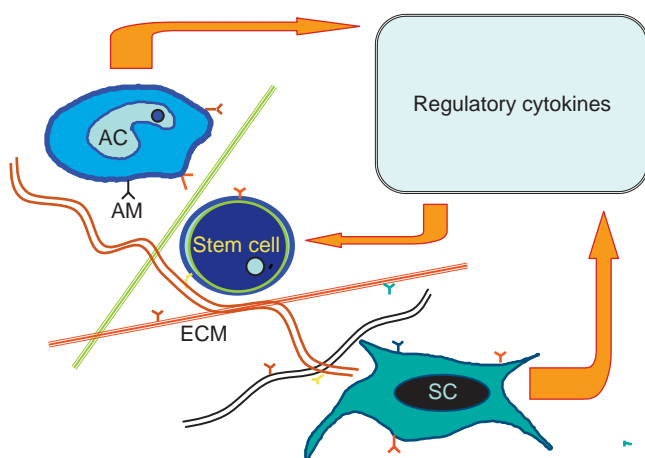


FIGURE 1.2 Cytokines released from accessory cells (AC) (e.g. macrophages, T-cells) and stromal cells have a regulatory effect on stem cells. The extracellular matrix (ECM) and adhesion molecules (AM) support cell–cell, cell–matrix, and cell–cytokine interactions.

Erythropoiesis apparently begins with the commitment of a small pool of pluripotent stem cells to a primitive committed cell to non-lymphoid lineages referred to as CFU-GEMM. GEMM stands for granulocytes, erythrocytes, macrophages, and megakaryocytes. The most primitive committed erythroid progenitor in humans is the erythroid burst-forming unit (BFU-E), which divides and forms subpopulations of erythroid colonies known as colony-forming units (CFU-E). This process requires a combination of cytokines, such as erythropoietin (EPO), Steel factor (SF; c-kit ligand), and interleukin-3 (IL-3) (Figure 1.3) [33, 35, 36]. EPO is necessary for the CFU-E formation and terminal differentiation of erythroid progenitors. SF has no erythroid colony-forming ability but has marked synergistic effects on BFU-E formation in the presence of EPO [36]. Similarly, IL-3 and granulocyte-macrophage colony stimulating factor (GM-CSF) enhance EPO-dependent erythropoiesis (Table 1.3). Proliferation and maturation of CFU-E leads to the formation of erythroblasts, more mature erythroid precursors, and eventually enucleated reticulocytes and erythrocytes. The entire process requires approximately 2 weeks. Except for the newborns, only erythrocytes and polychromatic erythrocytes (reticulocytes) are released into the blood circulation.

Myelopoiesis begins with the differentiation of a small population of pluripotent stem cells to CFU-GEMM and then to the committed primitive myeloid precursors, granulocyte/macrophage colony-forming units (CFU-GM) [37–40]. This process requires GM-CSF, SF, IL-3, and IL-6 (Figure 1.3). CFU-GM give rise to more mature colony-forming units CFU-G, CFU-M, CFU-Eo, and CFU-Baso which in turn differentiate into neutrophils, macrophages, eosinophils, and basophils, respectively (Figure 1.3) [39, 41].

The neutrophilic precursors in bone marrow consist of two major compartments: mitotic and post-mitotic. The mitotic compartment consists of cells that are able to proliferate, such as myeloblasts, promyelocytes, and myelocytes. The post-mitotic compartment includes metamyelocytes, bands, and segmented neutrophils, representing more differentiated cells with no proliferating capacity. The released

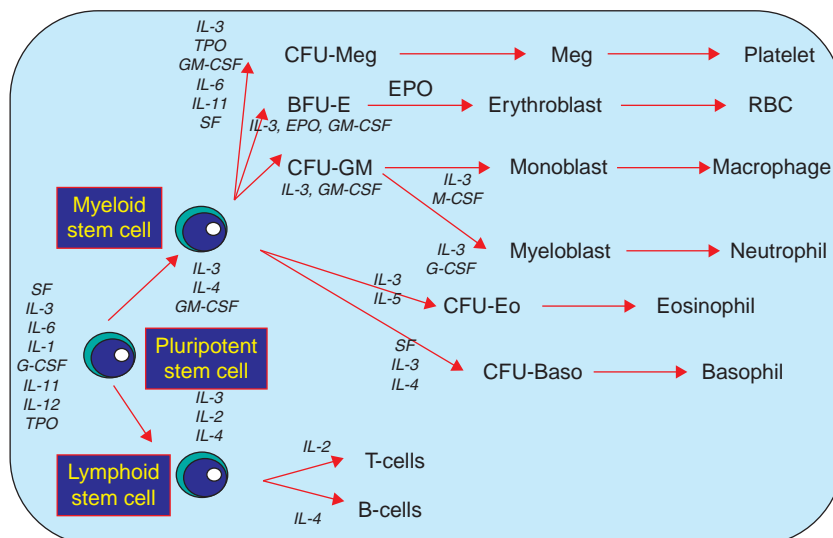


FIGURE 1.3 Current scheme of hematopoiesis demonstrating the differentiation of the multipotent stem cell to hematopoietic precursors and various levels of cytokine interaction.

granulocytes from bone marrow into the circulation consist of two components: the marginating pool and the circulating pool. These two components are in equilibrium. Granulocytes reside in the blood for an average of 10h and leave the circulation toward the inflammation sites in various tissues.

Eosinophil and basophil maturation appears to be analogous to neutrophil maturation, though the involved regulatory cytokines are different. GM-CSF and IL-5 play a major role in the development of the eosinophilic lineage, whereas SF, IL-3, IL-4, IL-9, and IL-10 regulate the basophilic development (Figure 1.3) [39, 41].

Monocytic maturation, assisted by GM-CSF and M-CSF, begins with the formation of monoblasts followed by promonocytes and monocytes. Monocytes are released into the circulation, and from there, they travel into various tissues and become various types of tissue macrophages (histiocytes) and dendritic cells. Pulmonary alveolar macrophages, hepatic Kupffer cells, pleural and peritoneal macrophages, osteoclasts, Langerhans and interdigitating dendritic cells in various tissues, and perhaps microglial cells in the central nervous system are all examples of cells derived from a monocytic lineage [42, 43].

Thrombopoiesis begins with maturation of CFU-GEMM into a colony-forming unit with a high proliferating response to cytokines (e.g. IL-1, IL-3, and IL-6) referred to as the high proliferative potential-colony-forming unit-megakaryocyte (HPP-CFU-MK) [44, 45]. The next step is the formation of a burst forming unit (BFU-MK), which is capable of producing numerous megakaryocytic colony-forming units (CFU-Meg). This process is regulated by SF, IL-3, GM-CSF plus thrombopoietin (TPO), and IL-11 (Figure 1.3) [45, 46]. Maturation of CFU-Meg leads to the formation of megakaryoblasts, megakaryocytes, and, eventually, platelets [45, 47].

Lymphopoiesis begins in the bone marrow with the committed lymphoid stem cells [25, 48–50]. The precise mechanism involved in the differentiation of HSCs into lymphoid precursors is not well understood. However, it is clear that the first step is the separation of B progenitor cells from non-B progenitor cells (T-cells and natural killer cells) [51–53]. This progression does not need interaction with exogenous antigen and therefore is considered the “antigen-independent” phase.

The development of B progenitor cells is influenced by a number of regulatory cytokines including IL-1, IL-2, IL-4, IL-10, and interferon gamma (Figure 1.3) [51, 54, 55]. The B-cell precursors in bone marrow are known as hematogones [56, 57]. These cells are found in small numbers in normal bone marrow (usually between 5% and 10% in young children and <5% in adults) but may increase in regenerating marrows.

T lymphocytes derive from the precursor lymphoid cells in the marrow (pre-thymic phase) under the influence of several cytokines, such as IL-1, IL-2, and IL-9, and then migrate to the thymus for further maturation [50–53, 58]. A subclass of large granular lymphocytes, natural killer (NK) cells, appears to share a common progenitor cell with T-cells in the marrow [59–62]. The NK-cells demonstrate HLA-nonrestricted cytotoxicity and release various regulatory cytokines, such as IL-1, IL-2, IL-4, and interferons.

Bone Marrow Examination

Bone marrow samples are obtained and prepared for pathologic evaluation in different ways, such as biopsies, clotted aspirated marrow particles, marrow smears, and touch preparations (Figures 1.4 and 1.5) [26, 63–67].

Bone marrow biopsy sections are evaluated for the estimation of bone marrow cellularity and for the identification of pathological processes, such as primary hematologic disorders, granulomatosis, amyloidosis, fibrosis, osteosclerosis, and metastasis. Biopsy sections are routinely stained with hematoxylin and eosin (H&E stain) (Figures 1.5 and 1.6). In addition, in some laboratories, sections are stained with periodic acid Schiff (PAS) technique. Bone marrow cellularity is defined as the percentage of the bone marrow areas occupied by cells (% cellularity = 100% area occupied by fat). Cellularity of the bone marrow varies depending on the location of the marrow sample and the age of the individual. For example, bone marrow cellularity is higher in the vertebrae than in the pelvic bone, and higher in the children than in the elderly. Bone marrow cellularity approaches 100% at birth and continues to decline approximately 10% for each decade of life. In a 50-year-old healthy person, the average bone marrow cellularity is about 50% (Figures 2.5 and 2.6).

Bone marrow clot sections (particle sections) are prepared from aspirated bone marrow and therefore are devoid of bone trabeculae. They only represent cells and lesions that are released by aspiration (Figures 1.4 and 1.5). Clot sections are routinely stained with H&E. Some laboratories may also use PAS stain.

Bone marrow smears are prepared by smearing the aspirated marrow over the glass slides. Marrow smears are usually stained with Wright's (or in some laboratories with Geimsa) stains (Figures 1.5–1.7). They are used primarily for the cytological evaluations, cellular details, maturation steps, differential count, and assessment of the myeloid:erythroid (M:E) ratio (normal range = 2–3). Differential counts reflect the percent of different hematopoietic cells in bone marrow smears. At least 200 cells are counted by randomly selected areas of a properly stained and adequately cellular marrow smear to calculate the differential count (Table 1.4). Marrow smears are also useful for special cytochemical stains and evaluation of the bone marrow iron stores.

Bone marrow touch (imprint) preparations are made by gently touching (pressing) the glass slides over the biopsy sample and are routinely stained with Wright's and/or Geimsa stains (Figures 1.4–1.6). Touch preparations most often are not optimal for morphologic evaluations, because their preparation creates significant artifacts. However, they are the only source of cytologic evaluation when bone marrow aspiration fails to yield (dry tap).

Morphologic Characteristics of Hematopoietic Cells

Granulocytic Series

Granulocytic series include neutrophilic, eosinophilic, basophilic, and mast cell lineages. The morphologic steps

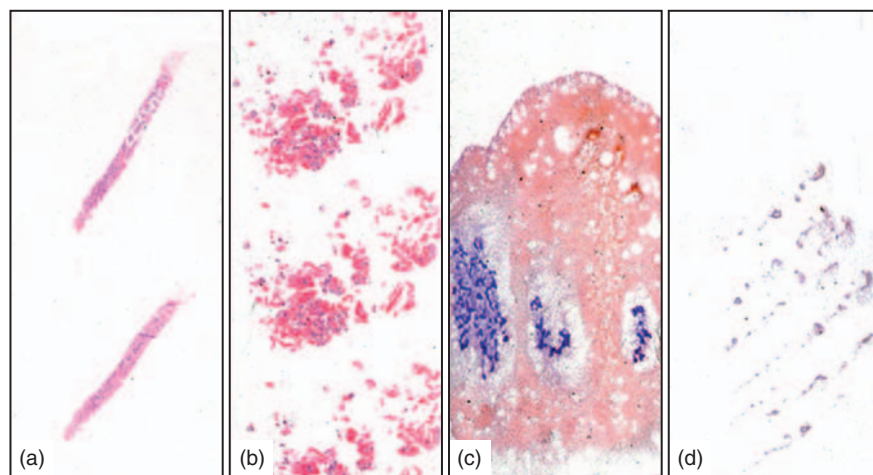


FIGURE 1.4 Representative examples of glass slide preparations of bone marrow biopsy (a), clot sections (b), bone marrow aspirate smear (c), and touch preparation (d).

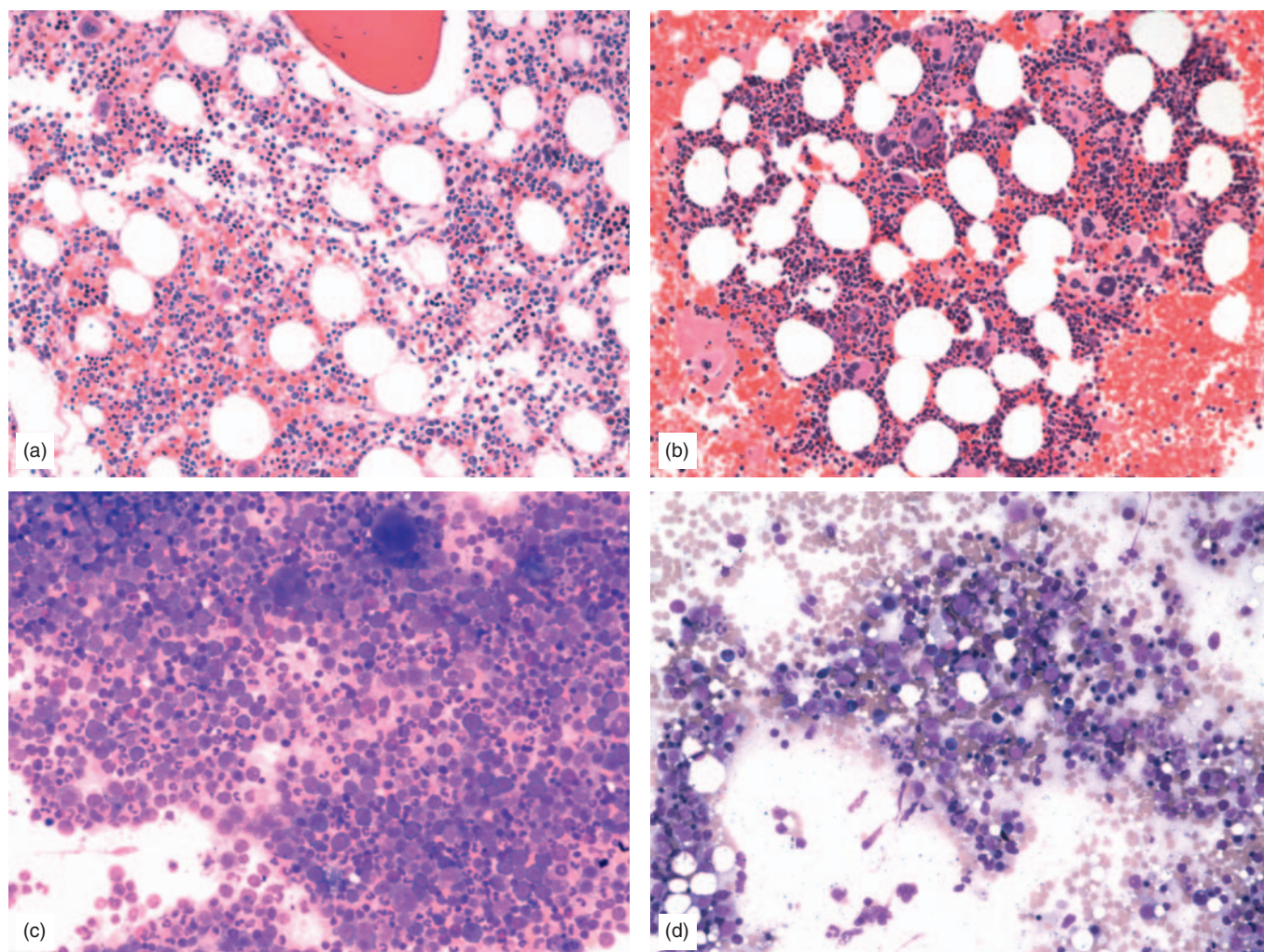


FIGURE 1.5 Bone marrow preparations: Biopsy section (a), clot section (b), aspirate smear (c), and touch preparation (d).

in the maturation process of the granulocytic series include myeloblast, promyelocyte, metamyelocyte, band, and segmented cell (Figures 1.7–1.9). During this process, the cytoplasmic:nuclear ratio increases, cytoplasm accumulates

lysosomal granules that are non-specific at first (primary granules, azurophilic granules) and become specific (secondary granules) later. The nuclear chromatin becomes coarser and denser, and the nucleoli appear less prominent

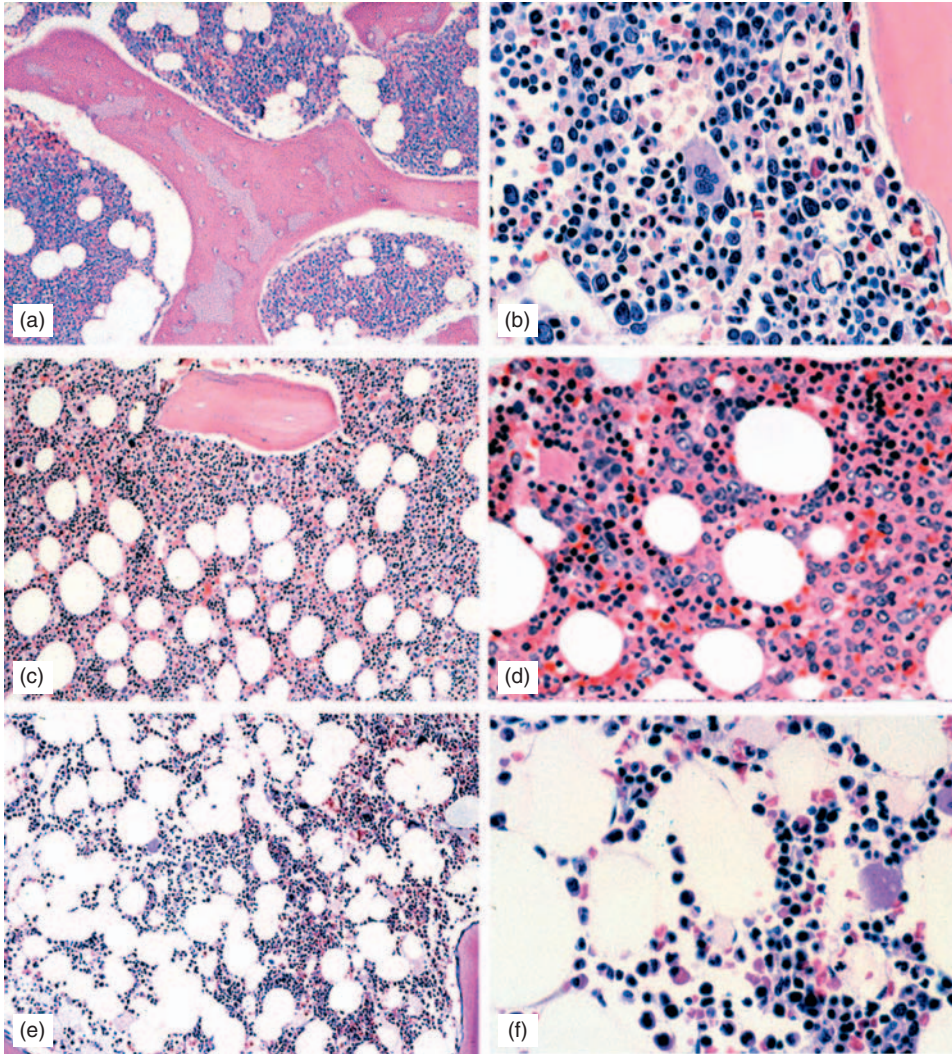


FIGURE 1.6 Bone marrow cellularity declines by age. (a) and (b); (c) and (d); and (e) and (f) are biopsy sections from 2-year, 55-year, and 75-year-old individuals, respectively. From Ref. [63] by permission.

and indistinct. The nuclear shape gradually changes from round/oval to kidney-shaped and segmented forms.

Myeloblasts are the earliest granulocytic precursors identified by morphologic evaluations. They range in size from 10 to 20 μm and are characterized by a high nuclear:cytoplasmic (N:C) ratio, a centrally located round or oval nucleus, finely dispersed chromatin and several nucleoli. Based on their cytoplasmic granules, myeloblasts are divided into three types (Figure 1.8) [68]:

Type I myeloblasts contain no cytoplasmic granules.

Type II myeloblasts contain <20 cytoplasmic azurophilic granules.

Type III myeloblasts contain >20 cytoplasmic azurophilic granules.

Myeloblasts are positive for CD13, CD33, and HLA-DR and may express CD117, CD34, and myeloperoxidase (MPO) [69, 70].

Promyelocytes are overall larger than myeloblasts, ranging from 13 to 25 μm in diameter. They carry more cytoplasm and contain larger quantities of azurophilic

granules than myeloblasts. They depict a perinuclear pale area (a well-developed Golgi system) and a round or oval nucleus, which is often eccentric. Type III myeloblasts and promyelocytes share overlapping morphologic features, and therefore their distinction at times is difficult (Figures 1.7 and 1.8). Myeloblasts are HLA-DR-positive and may express CD34, whereas promyelocytes are negative for HLA-DR and CD34. Promyelocytes are positive for CD13, CD33, and MPO and may express CD117 [69, 70].

Myelocytes are smaller than promyelocytes and are characterized by a reduced N:C ratio with ample granular cytoplasm containing both primary and secondary granules. At the myelocytic stage the production of primary granules stops and the synthesis of specific granules enhances. Myelocytes depict a round or oval nucleus with coarse chromatin and often lack distinct nucleoli (Figures 1.7 and 1.9). Myelocytes are positive for CD13, CD15, and MPO, and may express CD33. They are negative for CD34, CD117, and HLA-DR.

Metamyelocytes are slightly smaller than myelocytes and are characterized by abundant granular cytoplasm with predominance of specific granules, kidney-shaped or indented nucleus, coarser chromatin, and lack of distinct

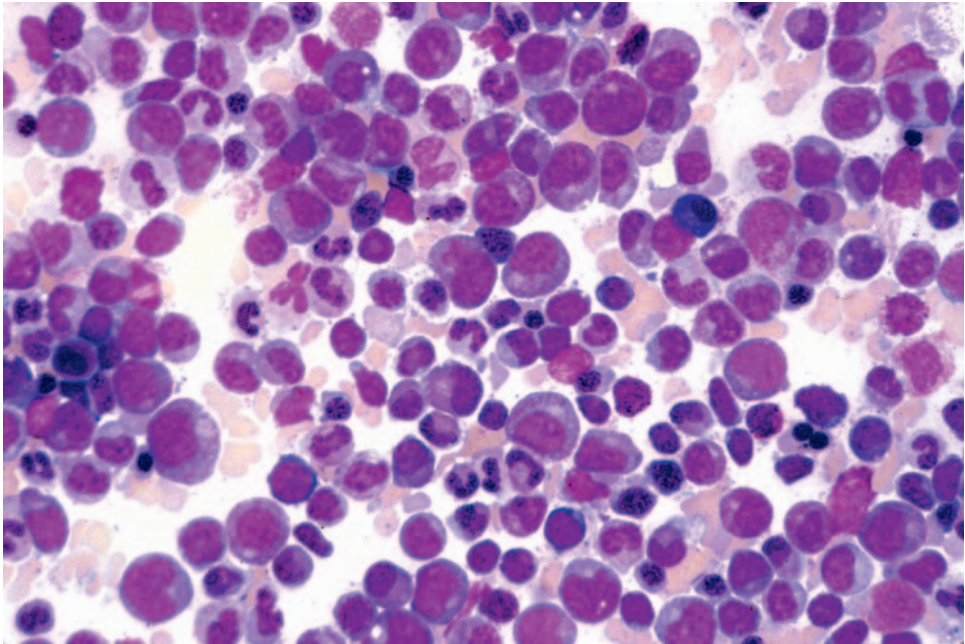


FIGURE 1.7 Bone marrow smears demonstrating myeloid cells in various stages of maturation. Scattered erythroid precursors and lymphocytes are also present.

TABLE 1.4 Approximate ranges of bone marrow differential counts in healthy persons.*

Cell type	Age	
	18 months	Adult
<i>Myeloid</i>		
Myeloblast	N/A	1–5
Promyelocyte	1–2	1–8
Neutrophilic series		
Myelocytes	2–4	5–19
Metamyelocyte	8–16	13–22
Band/Segs	14–25	21–40
Eosinophilic series	1.5–3.5	0.5–3
Basophilic series	<1	<1
Monocytic series	1–3	1–4
<i>Erythroid</i>		
Rubriblast	<1	0.5–2
Prorubricyte	0.5–1	1.5–6
Rubricyte	4–10	5–25
Metarubricyte	<1	2–20
Megakaryocyte	<1	0.5–2
Lymphocyte	40–42	3–20
Plasma cell	<1	0.5–2
M:E ratio	4–5:1	3–3.5:1

*References: Williams (1990), Brunning (1994), Bain (1996), Naeim (1998), and Greer (1999).

nucleoli (Figure 1.9). Metamyelocytes express CD13, CD15, and MPO and are negative for CD33, CD34, CD117, and HLA-DR.

Bands and **segmented cells** are the end stage cells in the granulocytic series and are distinguished by abundant cytoplasm with specific granules, lack of sparse primary granules, condensed nuclear chromatin with indistinct nucleolus, and nuclear lobulation or segmentation. Neutrophilic bands (stabs) are cells with bilobed nuclei with no filament formation, and neutrophilic segmented cells (Segs) demonstrate up to five distinct nuclear lobules (segments) connected to one another by filaments (Figure 1.9). Neutrophilic bands and segmented cells demonstrate alkaline phosphatase activity and are positive for CD11c, CD15, CD16, and MPO and may express CD13.

Other granulocytic lineages, such as *eosinophils* and *basophils*, undergo more or less similar differentiation steps. Mature eosinophils, unlike segmented neutrophils, usually have bilobed nuclei and are loaded with eosinophilic granules [71, 72]. Eosinophilic granules are larger than the neutrophilic granules (Figure 1.10). Mature basophils contain a large number of coarse basophilic granules and show less nuclear segmentation than the neutrophils (Figure 1.10) [73–75]. **Mast cells** appear to be closely related to the basophils by sharing certain characteristics, such as basophilic granules, IgE receptor, and histamine content [73, 75, 76]. However, mast cells live longer, are larger, have more abundant cytoplasm than basophils, and their nucleus is round, oval, or spindle-shaped without segmentation. Mast cell cytoplasmic granules are MPO negative and are more numerous and more variable in appearance than the granules in basophils (Figure 1.11). Mast cells express CD117 and tryptase [77, 78].

Monocytes and macrophages are derived from the same committed stem cells (CFU-GM) as the granulocytic cells [42, 43]. The maturation process in this lineage starts from *monoblast*, and then goes through *promonocyte*, *monocyte*, *macrophage (histiocyte)*, and *multinucleated giant cell* (such

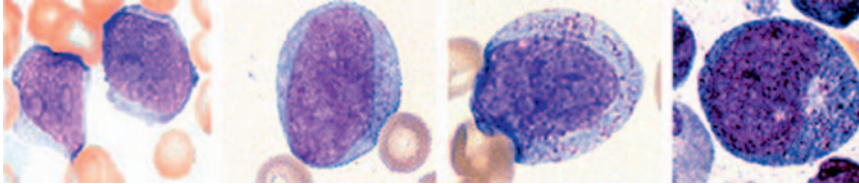


FIGURE 1.8 Left to right: myeloblast types I, II and III and a promyelocyte. From Ref. [63] by permission.

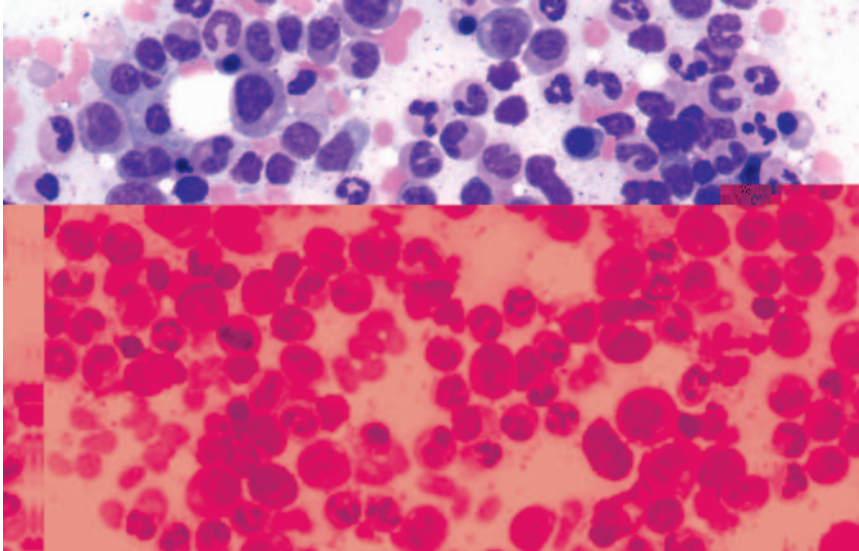


FIGURE 1.9 Bone marrow smears demonstrating myeloid cells in various stages of maturation.

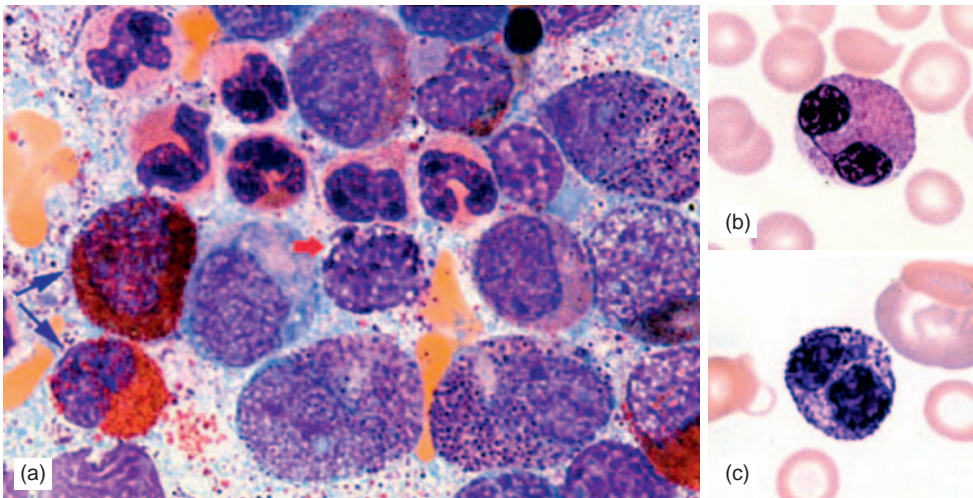


FIGURE 1.10 (a) A bone marrow smear showing granulocytic precursors including eosinophilic myelocytes (blue arrows) and a basophil (red arrow). An eosinophil (b) and a basophil (c) are demonstrated in blood smears. From Ref. [63] by permission.

as osteoclasts or giant cells in granulomas) (Figures 1.12 and 1.13). During the maturation process from monoblast to monocyte, nucleoli become folded, nuclear chromatin gets more condensed, nucleoli disappear, and cytoplasm acquires lysosomal granules (Figure 1.12). Monocytes are positive for CD13, CD14, CD15, CD64, CD11 (b and c), HLA-DR, and non-specific esterase, and may express CD33 and/or CD68 [79]. Monocytes are released from bone marrow into the blood circulation, and from there they migrate out into various tissues, and finally transform to soft tissue histiocytes (macrophages). Iron is stored in bone marrow macrophages as hemosiderin (insoluble aggregates) or less abundantly as ferritin (soluble). Prussian blue

(potassium ferrocyanide) stains hemosiderin as dark blue cytoplasmic granules (Figure 1.13a).

Dendritic cells are considered a subclass of histiocytic lineage and are primarily involved in antigen presentation to lymphocytes [80–83]. Dendritic cells, except for the follicular dendritic cells (FDCs), are derived from bone marrow stem cells. These cells are divided into two groups: Langerhans cells (LCs) (Figure 1.13c) and interdigitating dendritic cells (IDCs). LCs are primarily located in the skin and are characterized by the ultrastructural Birbeck granules and the expression of CD1a, CD4, S100, HLA-DR, and Langerin (CD207) [82]. Unlike macrophages, LCs are usually negative for CD68, non-specific esterase,

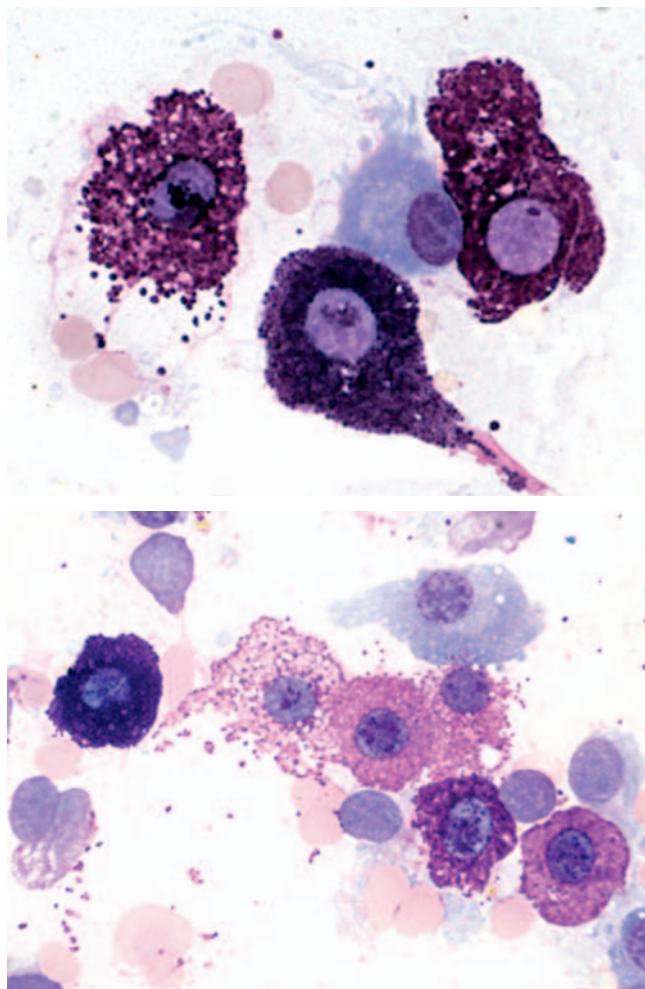


FIGURE 1.11 Bone marrow smears demonstrating mast cells with various amounts of cytoplasmic granules.

and lysozyme. IDCs are found in the lymphoid tissues and show immunophenotypic features similar to those of LCs, except for lack of CD1a and CD4 expression. The FDCs are derived from the mesenchymal cells in the follicular structures in the lymph nodes and express CD21 and CD35 [84, 85].

Erythroid Precursors

During the maturation process in erythropoiesis, cells gradually become smaller, the cytoplasmic:nuclear ratio increases, cytoplasm accumulates hemoglobin, the nuclear chromatin becomes denser and pyknotic, and the nucleoli appear less prominent and indistinct. The nucleus is eventually extruded from the cell, resulting in the development of polychromatophilic, and then mature red blood cells (RBCs) (Figures 1.14 and 1.15) [26, 64]. Erythroid precursors express several membrane-associated molecules, such as CD71, CD235 (glycophorin), CD238 (Kell blood group), CD240 (Rh blood group), and CD242.

Rubriblasts (erythroblast, pronormoblast) are the earliest morphologically distinguished erythroid precursors. They measure 15–30 μm in diameter and have a high

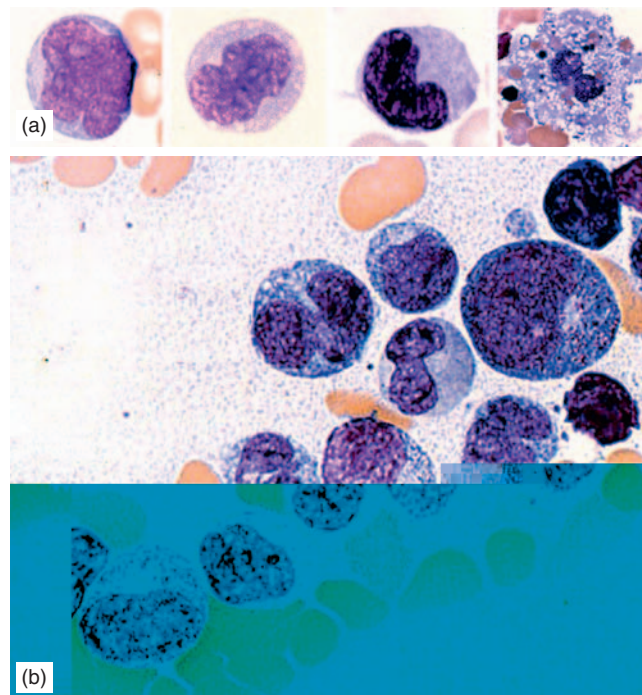


FIGURE 1.12 Left to right: (a) monocyte maturation from monoblast to promonocyte, monocyte, and macrophages. Bone marrow smear (b) showing several monocyte cells. From Ref. [63] by permission.

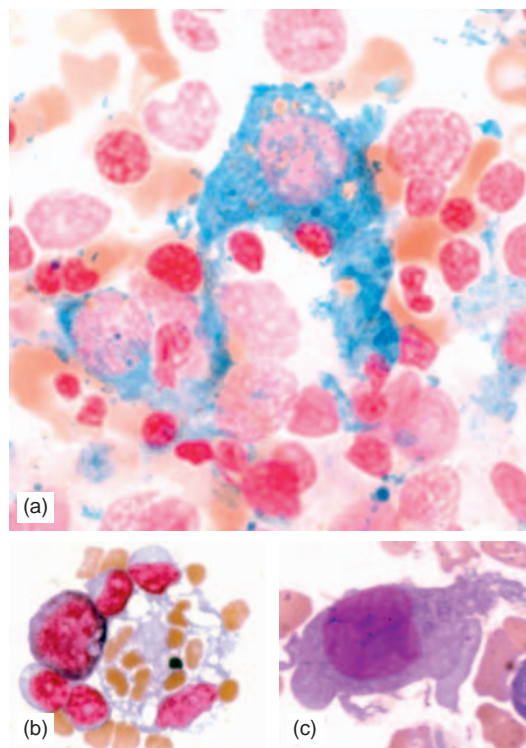


FIGURE 1.13 (a) A bone marrow clot section with iron stain showing iron-laden macrophages. (b) An erythrophagocytic macrophage surrounded by lymphoid cells and a rubriblast. (c) A Langerhans cell.

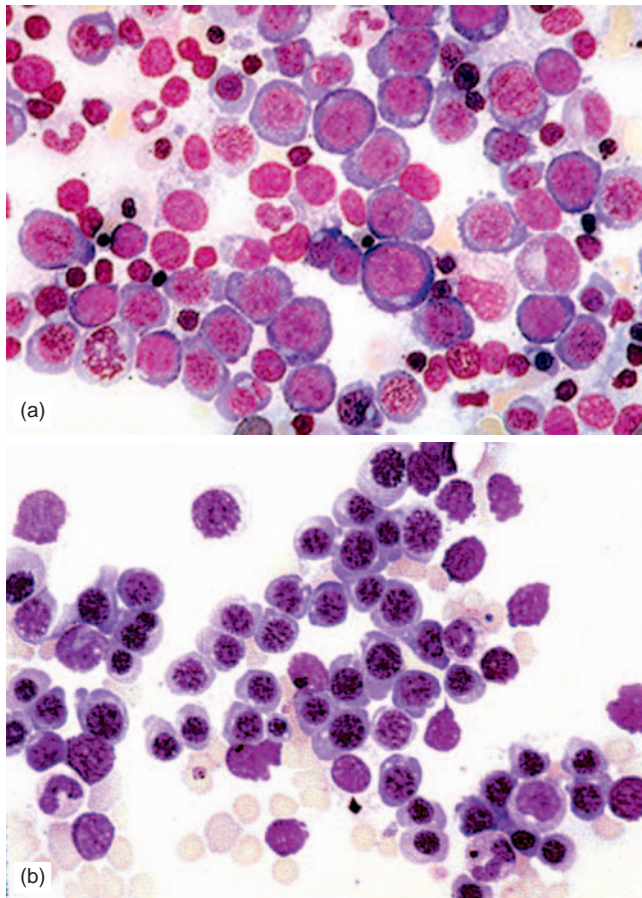


FIGURE 1.14 A bone marrow smear showing erythroid precursors of early (a) and intermediate (b) stages of maturation.

N:C ratio with a deep blue non-granular cytoplasm and a perinuclear pale area (the Golgi system). The nuclear chromatin is fine and one to two nucleoli are present. *Prorubricytes* (basophilic erythroblasts, basophilic normoblasts) are smaller and have more condensed nuclear chromatin than rubriblasts. They depict dark blue cytoplasm and indistinct nucleoli. Prorubricytes undergo three cell divisions and continue maturation to form *rubricytes* (polychromatophilic normoblasts), and subsequently *metarubricytes* (orthochromic normoblast) which are not able to divide but continue hemoglobin synthesis (Figure 1.15). Metarubricytes lose their nuclei and become polychromatophilic RBCs (reticulocytes). Reticulocytes gradually lose their ribosomes (in 1–2 days) and become mature RBCs.

Platelet Precursors

Megakaryoblasts (promegakaryoblasts, group 1 megakaryocytes) are the earliest morphologically identifiable platelet precursors (Figure 1.16) [86, 87]. Megakaryoblasts undergo endomitosis (nuclear division without cytoplasmic division) once or twice and become *promegakaryocytes* (group II megakaryocytes). Endomitosis continues, cells become larger, the nuclear lobulation and volume increase, and

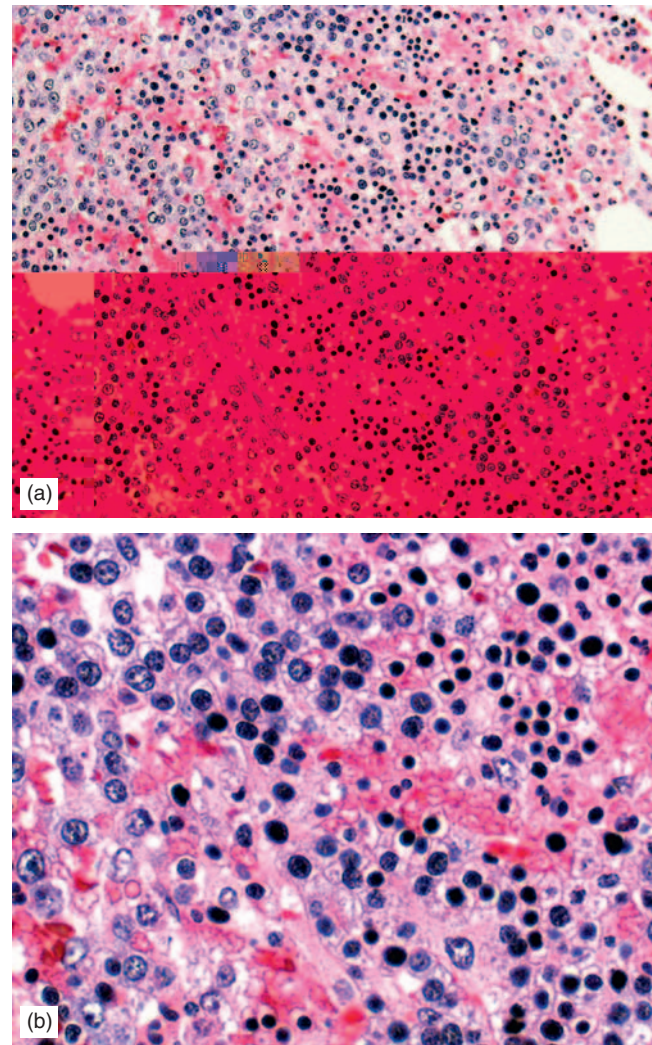


FIGURE 1.15 A bone marrow clot section (a, low power and b, high power) showing erythroid precursors at various stages of maturation.

the end result is the formation of *granular megakaryocytes* (group III megakaryocytes) which are able to release platelets (Figure 1.16) [45, 47, 88, 89]. Granular megakaryocytes are the largest hematopoietic cells in the bone marrow. The duration from formation of megakaryoblasts to platelet production is about 1 week [89, 90]. Platelets are released into the bloodstream with a proportion (approximately one-third) pooled in the spleen. Their average life span is about 8–10 days. Cells from megakaryocytic lineage express CD41, CD42, CD31, CD61, and factor VIII [90, 91].

Lymphoid Lineage

Lymphocytes, similar to the other hematopoietic cells, are derived from the multipotent stem cells [48, 49, 92, 93]. Lymphoblasts, the earliest morphologically identifiable lymphoid cells, have a high N:C ratio with a narrow rim of dark blue non-granular cytoplasm, a round or oval nucleus with fine chromatin and one to two nucleoli. Mature lymphocytes are slightly larger than erythrocytes

and are characterized by scanty blue cytoplasm, round nucleus, coarse chromatin, and inconspicuous nucleolus (Figure 1.17a). They may be of B- or T-cell origin. A variable proportion of lymphocytes are larger with abundant cytoplasm and cytoplasmic azurophilic granules. These *large granular lymphocytes* (LGL) are more frequently identified in normal blood smears than bone marrow smears and often express CD16, CD56, and/or CD57 molecules (Figure 1.17b) [61, 95]. They are of two types: NK-cells and cytotoxic T-cells. NK-cells are negative for surface CD3 and show no T-cell receptor (TCR) gene rearrangement but may express CD8, and cytotoxic T-cells express CD3 and CD8 and show *TCR* gene rearrangement [94–96].

Prolymphocytes are larger than lymphocytes (more cytoplasm and a larger nucleus), display a coarse chromatin, and often show a prominent nucleolus. They are either of B- or T-cell origin (Figure 1.17c).

Activated lymphocytes are transformed B-, T-, or NK-cells. These are large cells with abundant cytoplasm and a highly polymorphic nuclear morphology (Figure 1.17d). They are more frequently identified in blood smears than marrow smears.

Hematogones represent normal bone marrow precursor B-cells. These cells consist of a heterogeneous population [56, 57]. The earlier forms (stage 1 hematogones) often express TdT, CD34, CD10, and CD19. The more mature

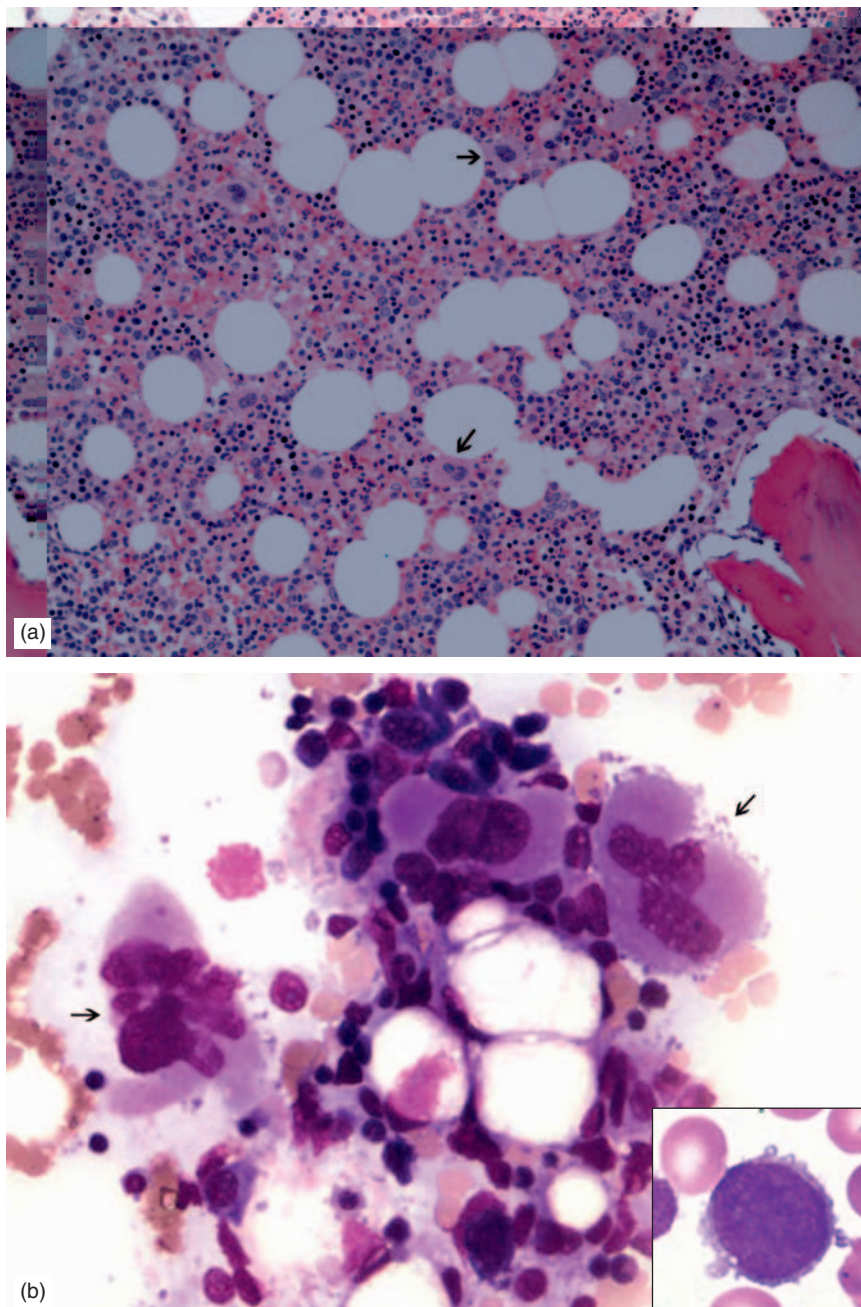


FIGURE 1.16 The megakaryocytic lineage: (a) bone marrow biopsy section demonstrating two megakaryocytes, several eosinophils, and numerous erythroid and neutrophilic precursors; (b) bone marrow smear demonstrating megakaryocytes. A megakaryoblast with cytoplasmic budding is demonstrated in the inset.

forms (stage 2 hematogones) lack the CD34 and TdT expression. These cells may morphologically resemble lymphoblasts but usually show somewhat denser chromatin and absent or inconspicuous nucleoli (Figures 1.18 and 1.19). Hematogones display a distinctive pattern in SSC versus CD45 studies by flow cytometry (Figure 1.20). They are CD45^{dim} and appear as the tail of the mature lymphocytes (CD45^{strong}). Hematogones account for about 5–10% of the bone marrow cells in children and <5% of the bone marrow cells in adults.

Plasma cells are the end product of the B-cell lineage and are characterized by abundant dark blue cytoplasm, a perinuclear pale area (Golgi system), and an eccentric nucleus

with coarse chromatin (cartwheel appearance) (Figure 1.21). Plasma cells may show small cytoplasmic vacuoles (Mott or morula cells), or large eosinophilic cytoplasmic inclusions (Russell bodies) or nuclear inclusions (Dutcher bodies) (Figure 1.22). Russell bodies and Dutcher bodies are more often seen in plasma cell disorders than in normal plasma cells. The vacuoles and inclusions contain immunoglobulin. Rarely, ovoid-, angular-, or rod-shaped immunoglobulin crystals are found in plasma cells. Cell-membrane-associated molecules CD19, CD38, CD138, CD79b, and sometimes CD117 are expressed by plasma cells [97–99].

Lymphoid aggregates are relatively common findings in bone marrow sections, particularly in the elderly. They are well-defined round or oval structures that are randomly distributed in the marrow, usually in close association with small blood vessels and apart from bone trabeculae. They primarily consist of small mature lymphocytes comprising a mixture of B- and T-cells (Figure 1.23). Scattered macrophages, eosinophils, and plasma cells may also be present within or around the lymphoid aggregates.

Other Bone Marrow Cells

Osteoblasts are derived from a multipotent mesenchymal stem cell, a lineage different from that of the HSC [26, 64]. These cells are elongated or oval cells that contain an eccentric round or oval nucleus and one or more nucleoli. Osteoblasts may resemble plasma cells, except that they are larger, their Golgi are not as close to the nucleus, and their nuclear chromatin is finer than that of plasma cells. Osteoblasts in biopsy sections are located along the bone trabeculae, and in bone marrow smears usually appear as individual or small cluster of cells (Figure 1.24). Osteoblasts release a number of matrix molecules and cytokines, such as collagen type 1, proteoglycans, osteoid, GM-CSF, M-CSF, and IL-6 [14, 100].

Osteoclasts are derived from the monocytic lineage [101, 102]. They are multinucleated giant cells involved in

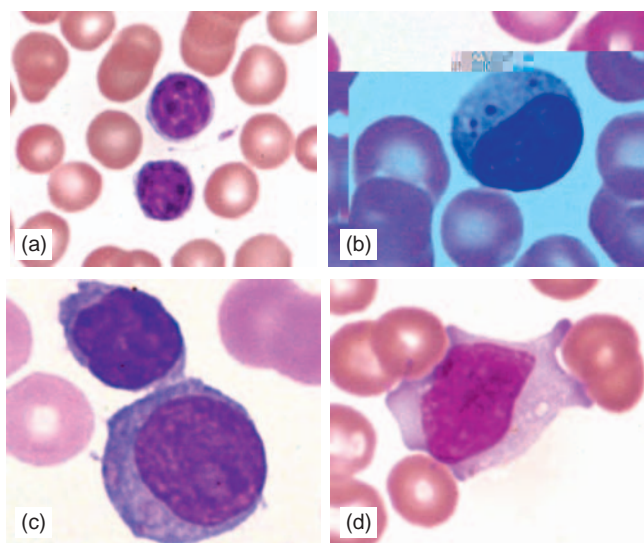


FIGURE 1.17 Examples of various lymphoid cells: (a) mature lymphocytes, (b) a large granular lymphocyte, (c) prolymphocytes, and (d) a reactive lymphocyte.

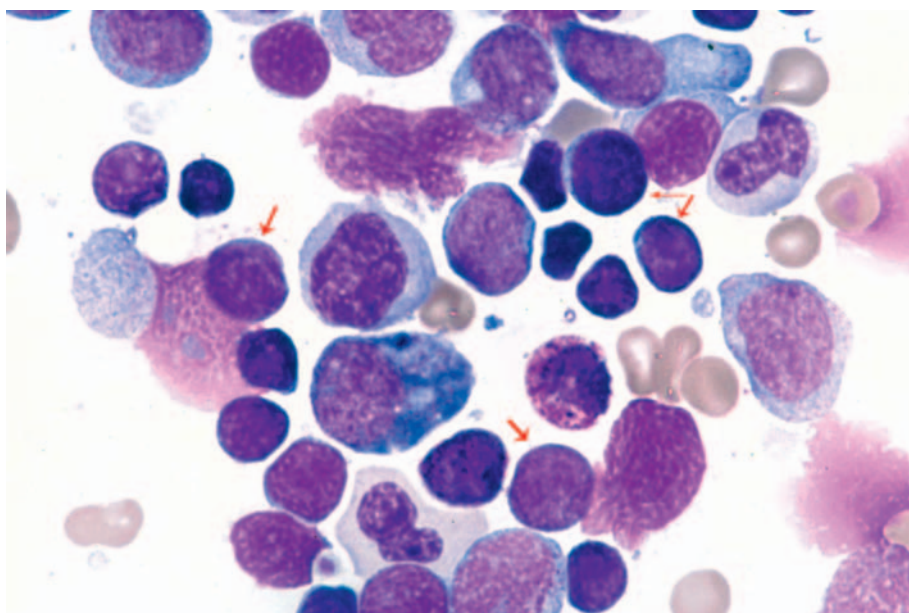


FIGURE 1.18 Several hematogones (arrows) are presented in this bone marrow smear.

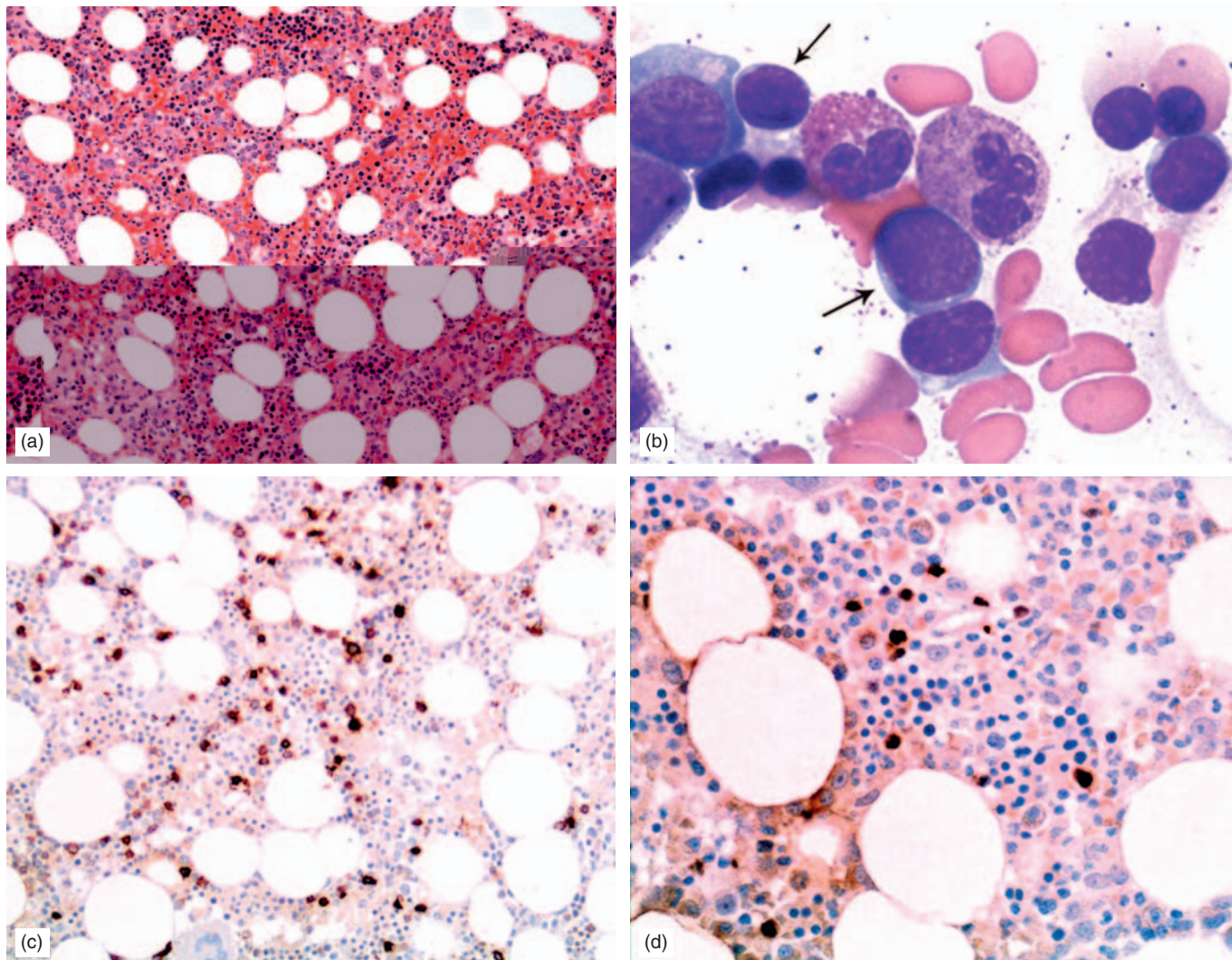


FIGURE 1.19 (a) An unremarkable bone marrow biopsy section showing progressive multilineage maturation. (b) A bone marrow smear demonstrating hematogones (arrows) and an eosinophil. (c) Scattered CD20-positive B-cells. (d) Rare TdT-positive cells which may represent early hematogones.

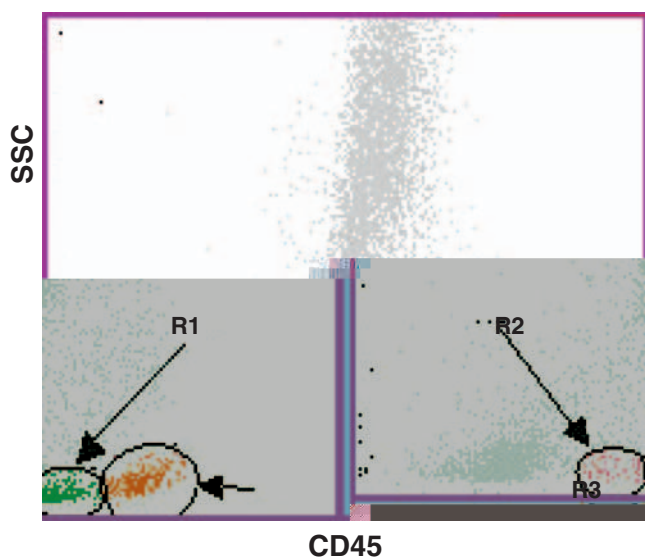


FIGURE 1.20 Flow cytometry. The sidescatter (SSC)/CD45 dot plot of a bone marrow sample demonstrates aggregates of early (R1) and late (R2) hematogones, and mature lymphocytes (R3).

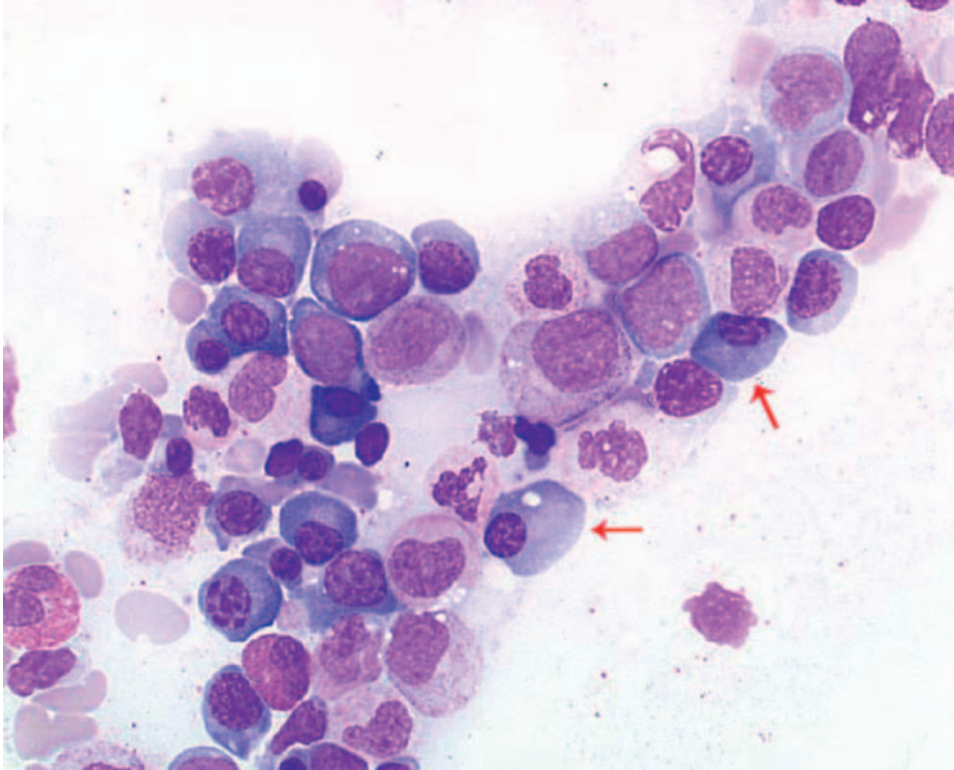


FIGURE 1.21 A bone marrow smear demonstrating several plasma cells (arrows).

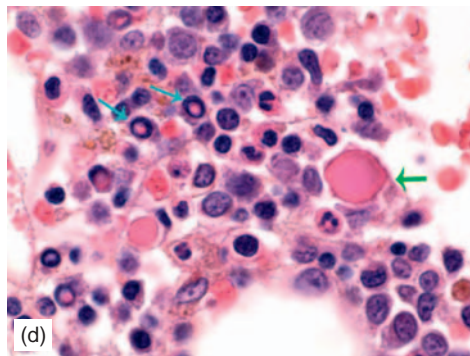
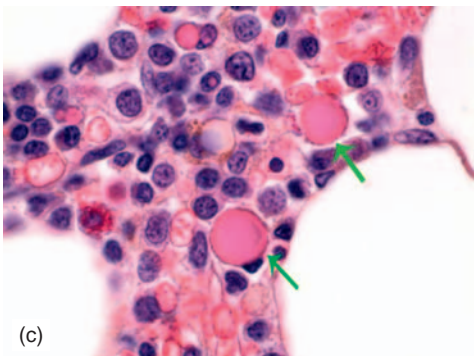
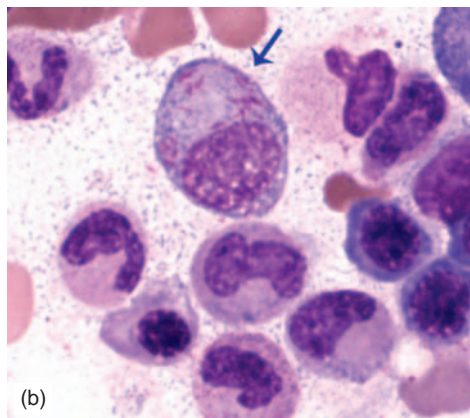
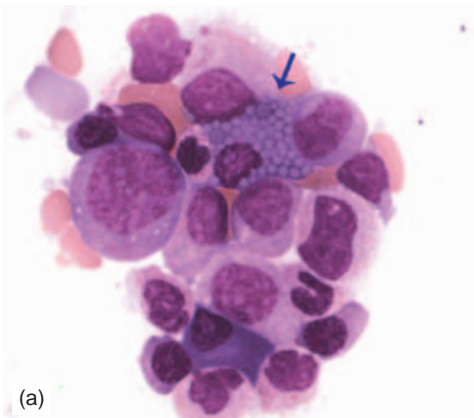


FIGURE 1.22 Plasma cells with inclusions: (a) a grape-like plasma cell (Mott cell), (b) plasma cells with cytoplasmic rod-like Ig crystals, (c) cytoplasmic Ig inclusions (Russell bodies), and (d) Russell bodies (green arrow) and nuclear Ig inclusions (Dutcher bodies) (blue arrows).

bone resorption and remodeling (Figure 1.25). They have abundant cytoplasm which contains numerous azurophilic granules. They are found along the bone trabeculae and may resemble megakaryocytes, except they have multiple separated nuclei that are uniform in size. Osteoclasts

are frequently observed in bone marrow of patients with hyperparathyroidism, chronic renal failure, and Paget's disease.

Adipocytes (fat cells, lipocytes) are mesenchymal-derived cells with abundant fat-laden cytoplasm and a

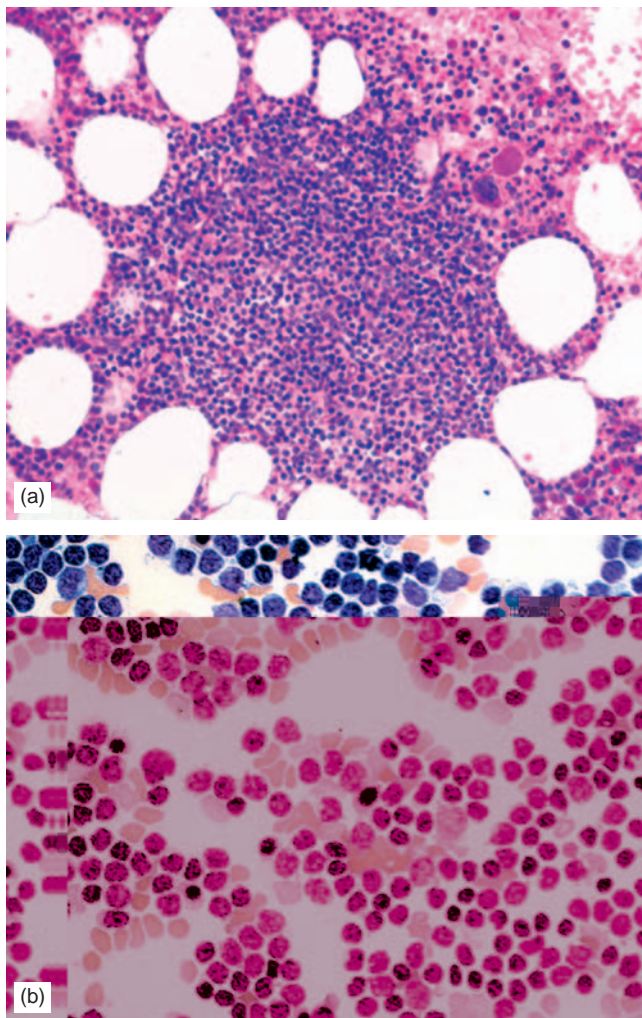


FIGURE 1.23 A well-defined lymphoid aggregate is shown from a bone marrow biopsy section (a) and numerous small mature lymphocytes are present in a bone marrow smear (b).

small nucleus often pushed toward the cell membrane (Figure 1.26) [103]. Bone marrow adipocytes can be rapidly replaced by hematopoietic tissue when there is a need for increased hematopoiesis.

Fibroblast-like cells and **endothelial cells** support the wall of the bone marrow sinuses and build the framework of the marrow stroma that supports the hematopoietic cells [104]. These are usually elongated or polygonal cells (15–30 μm) with variable amount of pale cytoplasm and round, oval, or folded nuclei. Their nuclear chromatin is fine and they may depict one or more nucleoli. Fibroblast-like cells support proliferation of myeloid and lymphoid progenitor cells. They are negative for CD33 and CD34 but may express CD10 [105]. Endothelial cells are involved in the regulation of homing and trafficking of the hematopoietic cells, as well as proliferation and differentiation of hematopoietic precursors (Figure 1.26). They express CD31, CD34, and CD146 and carry various receptors, such as receptors for IL-3, EPO, and SF [106,107].

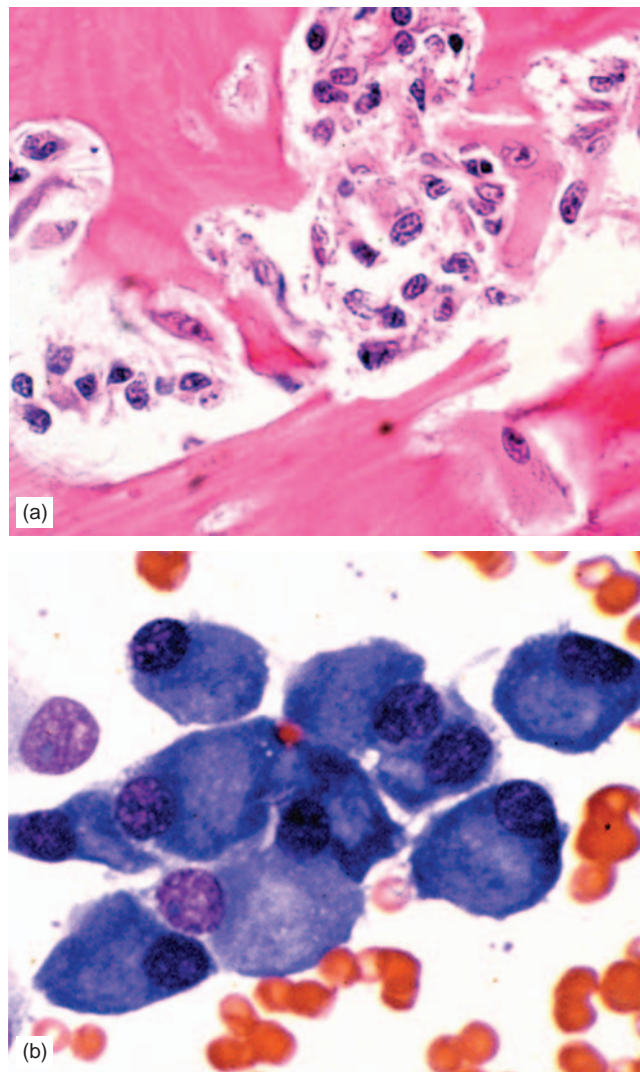


FIGURE 1.24 Numerous osteoblasts are demonstrated in a bone marrow biopsy section (a) and a bone marrow smear (b).

Blood Smear Examination

Morphologic evaluation of blood smear is important in routine hematology work-up, because unremarkable CBC results by automated instruments may not necessarily reflect normal hematopoiesis [108, 109]. For example, in hereditary spherocytosis, lead poisoning, or malaria, the CBC may be within normal limits, but the peripheral blood smears show spherocytes, basophilic stippling, or RBC-containing parasites, respectively. Blood smears should be thin, evenly distributed over the glass slides and quickly air-dried and stained (Wright's stain is the most popular stain).

RBC Morphology

In normal conditions, red cells are relatively uniform in shape and size and contain no inclusions. They are normocytic (an average of 7–8 μm in diameter) and normochromic (the pale central area less than 1/2 of the RBC diameter) (Figures 1.27 and 1.28) [110]. One to two percent of erythrocytes are larger and polychromatophilic (bluish-red)

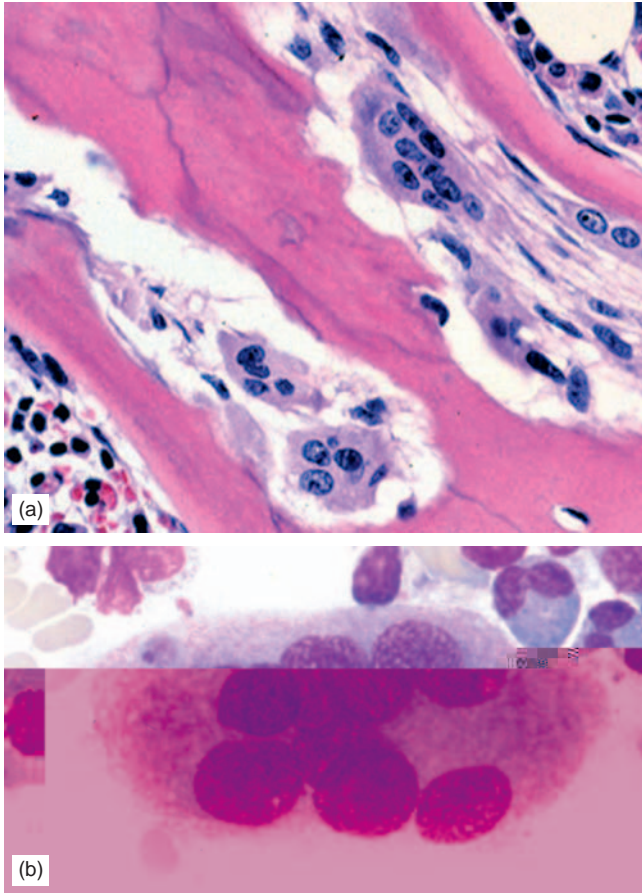


FIGURE 1.25 (a) Numerous osteoclasts are demonstrated in a bone marrow biopsy section. (b) An osteoclast with multiple separated nuclei and finely granular cytoplasm is shown.

(Figure 1.29). These represent reticulocytes [111]. Except in newborns, nucleated red cells are not normally found in peripheral blood.

Leukocyte Morphology

In normal conditions, peripheral blood smears show various proportions of neutrophilic segmented cells (Segs) and bands (stabs), lymphocytes, monocytes, eosinophils, and basophils (Figures 1.27–1.29). The white blood cell (WBC) count ranges from 3 to 10×10^3 cells/ μL with a differential count shown in Table 1.5.

Certain conditions such as exercise, emotional disturbances, menstruation, anesthesia, convulsive seizures, and electric shock may be associated with a transient neutrophilic granulocytosis. This is due to the demargination of the neutrophilic granulocytes and their release into the circulating pool. The presence of immature leukocytes in the peripheral blood should be considered abnormal.

Platelet Morphology

Platelets are the end products of the megakaryocytic lineage and are released into the circulation as cytoplasmic fragments of granular megakaryocytes. They are the smallest

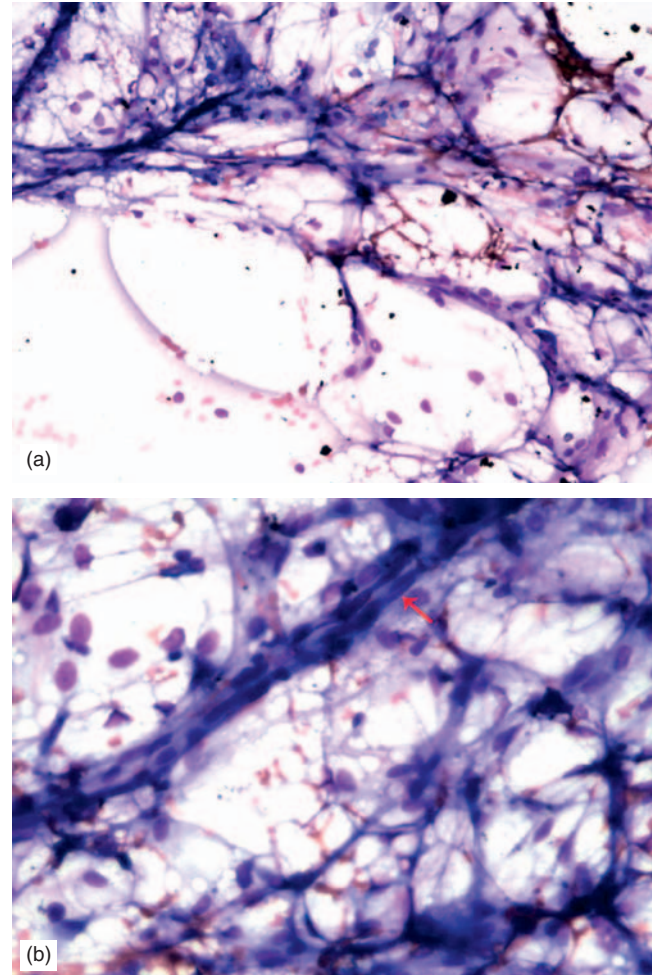


FIGURE 1.26 Bone marrow smears showing adipose tissue and stromal cells. A collapsed capillary (arrow), lined by endothelial cells, is present in (b).

hematopoietic elements (measuring $2\text{--}4\mu\text{m}$ in diameter), with a count ranging from $150,000$ to $400,000/\mu\text{L}$ (Figures 1.27–1.29). A rough estimate of the platelet count is calculated in wedge smear preparations by the number of platelets per oil-immersion field $\times 20,000$. Approximately $7\text{--}21$ platelets are found per $100 \times$ oil-immersion field in an evenly distributed normal blood smear. Anti-coagulants or agglutinins (IgM or IgG) which are found in patients with autoimmune disorders, chronic liver disease, or malignancy may cause platelet aggregation.

STRUCTURE AND FUNCTION OF THE SPLEEN

The spleen represents the largest filter of the blood circulation in our body. In normal conditions, it weighs between 75 and 200g and has a deep indentation (the hilum), where blood vessels enter and leave. The spleen is surrounded by a fibrous capsule with many trabeculae radiating from the

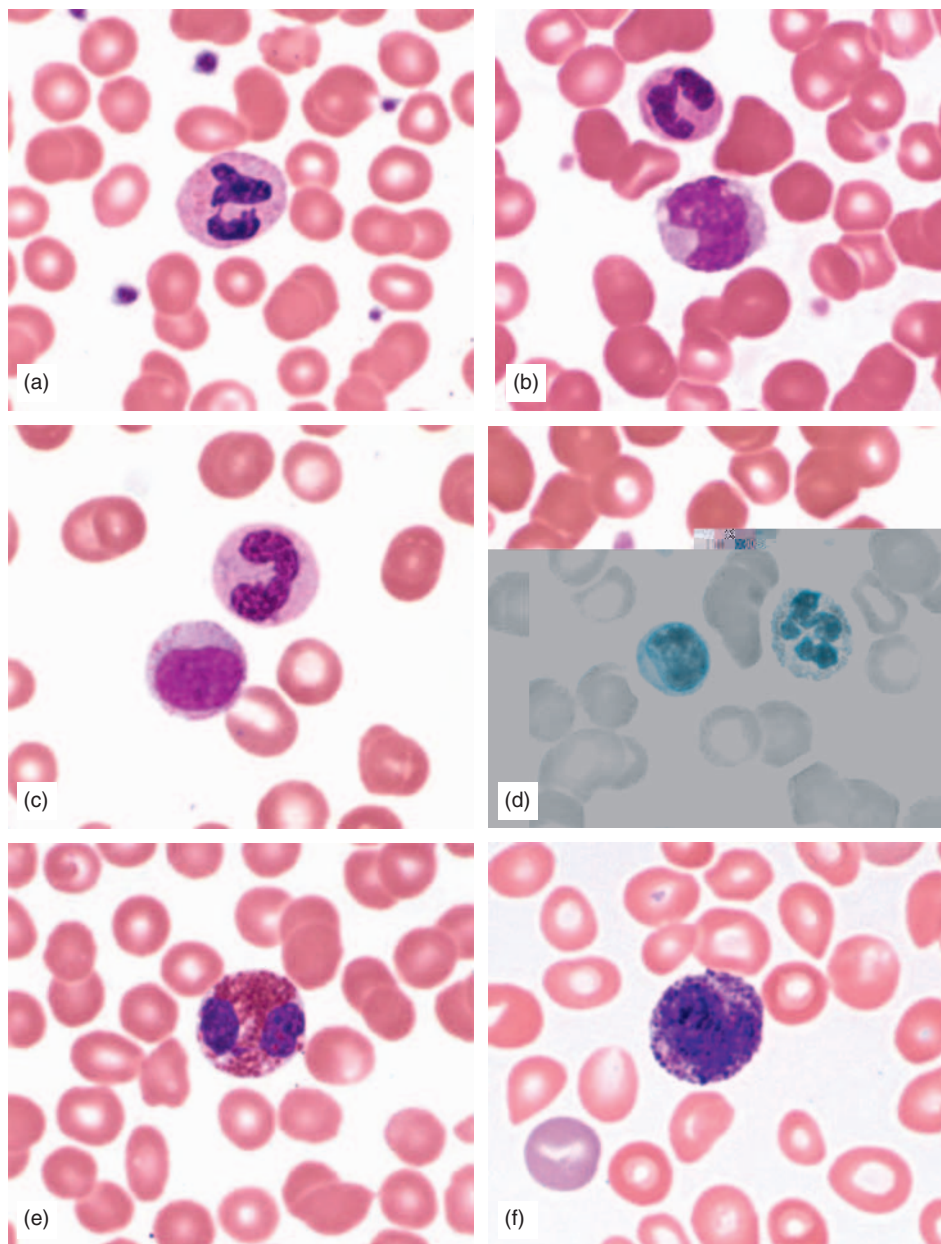


FIGURE 1.27 Blood smears demonstrating segmented neutrophils (a, b, and d), monocytes (b and d), a large granular lymphocyte (c), a lymphocyte (d), an eosinophil (e), and a basophil (f). Platelets are present in (a, b, and d).

hilum and from the internal surface of the capsule into the splenic parenchyma. The splenic artery branches into the trabecular arteries and these branches in turn give off smaller branches that leave the trabeculae and are called central arteries. Central arteries run through the splenic lymphoid tissue (white pulp) and extend to the marginal zone and the red pulp. Therefore, the splenic parenchyma consists of three distinct components: the white pulp, the marginal zone, and the red pulp (Figures 1.30 and 1.31) [112, 115].

The White Pulp

The white pulp consists of lymphoid structures organized in B- and T-cell zones (Figures 1.30 and 1.31) [116–118]. The T-cell zone is represented by the periarteriolar lymphoid sheath, primarily consisting of tightly packed lymphocytes

and the presence of IDCs. The T-cells interact with the dendritic cells and passing B lymphocytes. The B-cell zone consists of follicles, which are structurally similar to the follicular structures in the lymph nodes (see lymph node structure later). The follicles are separated from the marginal zone by a densely packed mantle zone and frequently contain germinal centers consisting of large blast-like lymphocytes (centroblasts), smaller lymphocytes (centrocytes), FDCs, and scattered macrophages. Follicles are the place for clonal expansion of activated B-cells, leading to isotype switching and somatic hypermutation.

The Marginal Zone

The marginal zone is the transit area for cells that are leaving the bloodstream and entering the white pulp (Figures

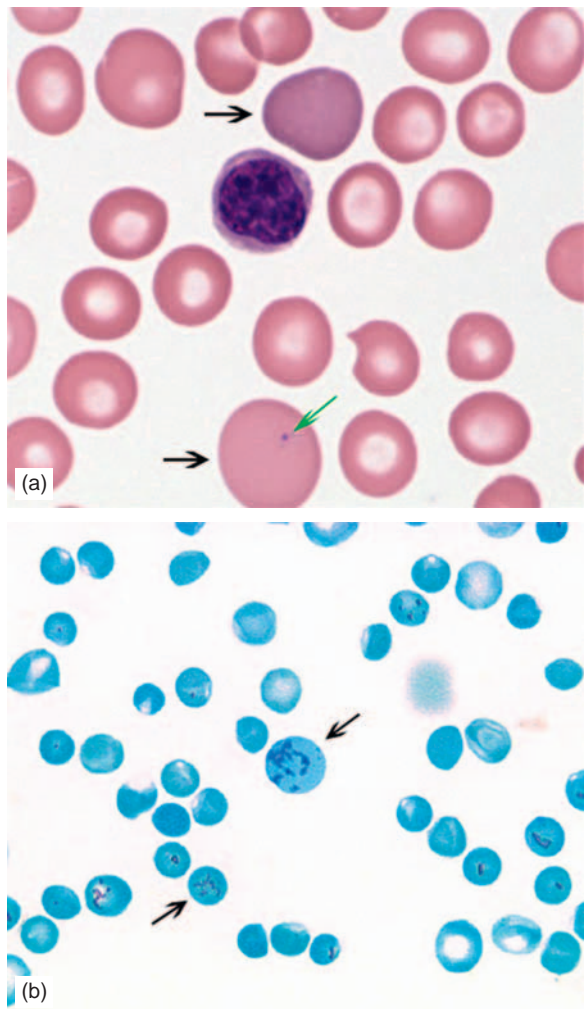


FIGURE 1.28 (a) Blood smear showing polychromatophilic red cells (black arrows) and a Howel-Jolly body (green arrow). (b) Reticulocytes are demonstrated by a supravital stain (arrows).

1.30 and 1.31) [116–120]. However, a large number of cells, such as macrophages, B-cells, and dendritic cells, reside in the marginal zones in order to regulate and facilitate the back and forth transit flow of cells between the blood and the white pulp. The marginal zone macrophages are of two subtypes: the outer ring macrophages, which are close to the blood vessels, and the inner ring macrophages, which are in the proximity of the white pulp. In between the inner and the outer rings, marginal zone B-cells and a subset of dendritic cells reside. Marginal zone B-cells are medium- to large-sized cells with pale cytoplasm and irregular nuclei, resembling monocytes. That is why they were originally called monocytoïd B lymphocytes. Because of the presence of variable amounts of cytoplasm, they show nuclear spacing in sections and appear lighter in color and less dense compared to the cells present in the mantle zone. The marginal zone B-cells do not express CD5, CD10, and CD23. They have mutated Ig-V genes and express surface IgM and IgD.

TABLE 1.5 The range of WBC differential counts in normal adults.

Cell type	Range (%)
<i>Granulocytes</i>	
Segs	33–72
Bands	0–13
Eosinophils	0–6
Basophils	0–3
<i>Lymphocytes</i>	16–48
<i>Monocytes</i>	1–13

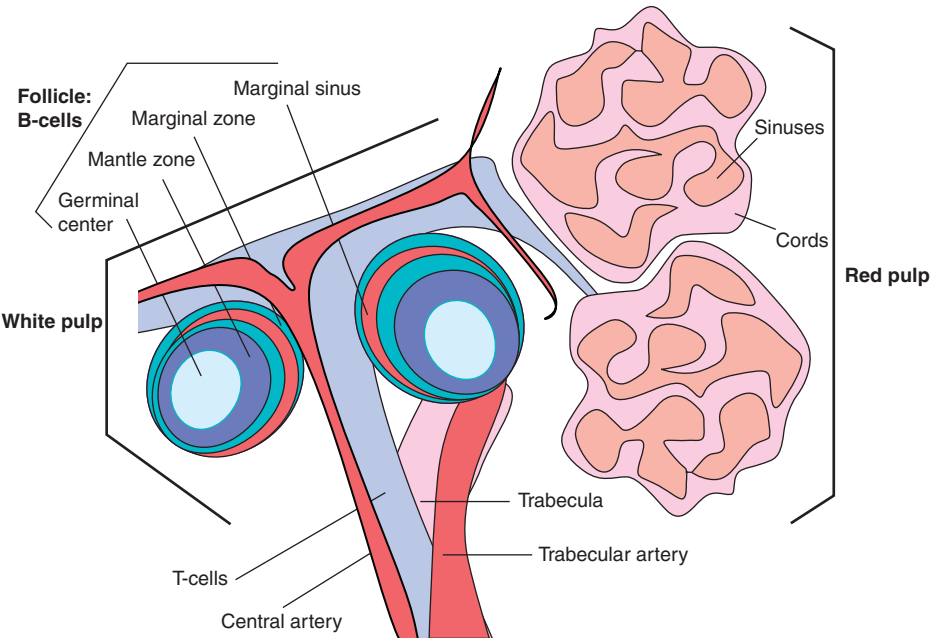


FIGURE 1.29 Schematic of a spleen demonstrating the white pulp, the red pulp, and the marginal zone. Adapted from Greer JP, et al. (2004). *Wintrob's Clinical Hematology*, 11th ed., Williams & Wilkins Lippincott.

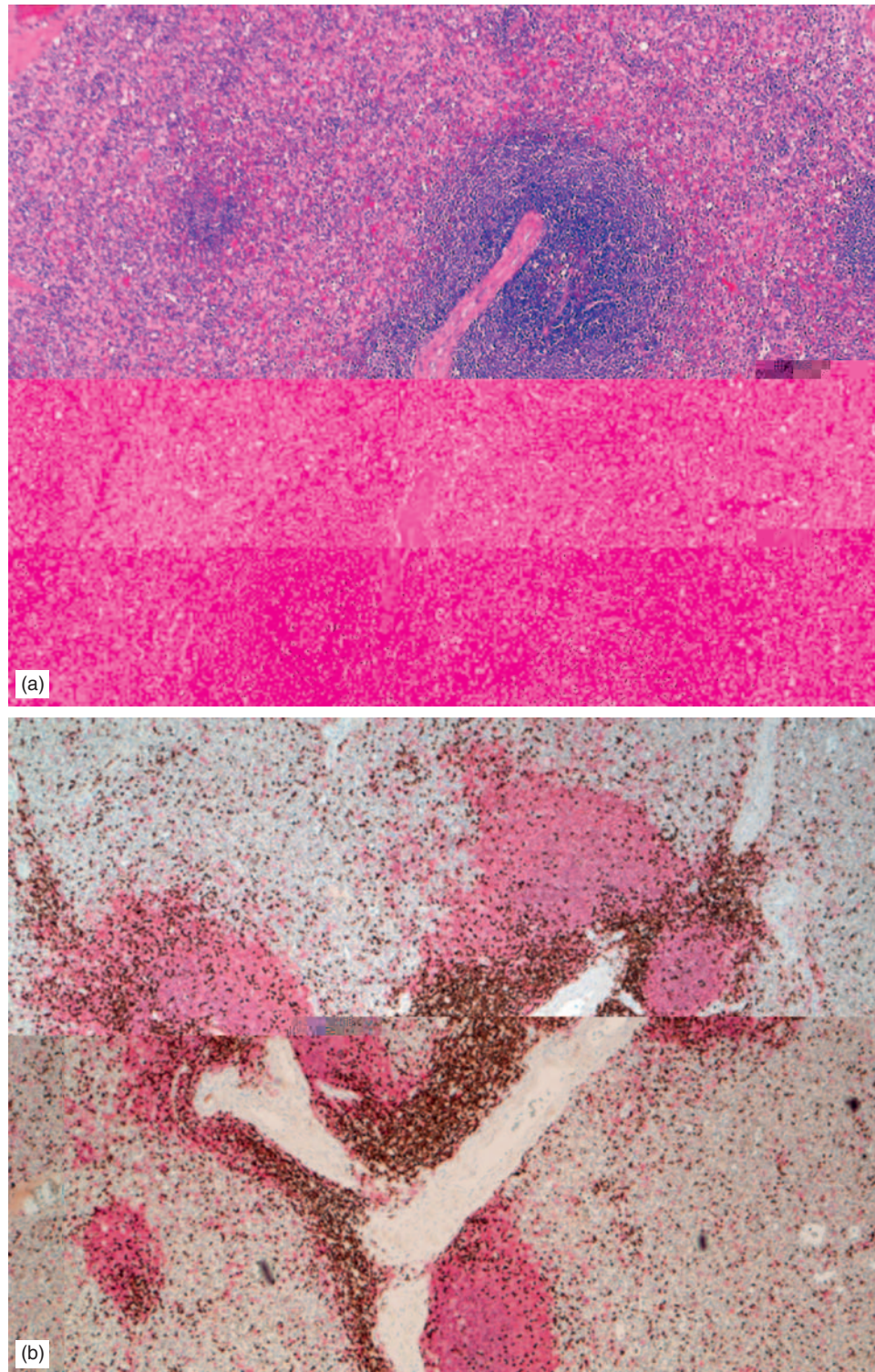


FIGURE 1.30 (a) White pulp and red pulp regions of the spleen are demonstrated in an H&E section. (b) Dual immunohistochemical staining for CD3 (brown) and CD20 (red) demonstrates T- and B-cell areas, respectively.

The marginal zone is a place where the blood-borne pathogens are challenged by the adaptive immune system. Numerous arteriolar branches are present in this region, some with funnel-shaped orifices. These funnel-shaped orifices facilitate the release of arterial content into the mantle

zone. However, the marginal zone is devoid of sinuses. Macrophages with their specific pattern-recognition receptors can effectively take up the pathogens and also activate the marginal zone B-cells and dendritic cells. Entry of activated marginal zone B lymphocytes and dendritic cells

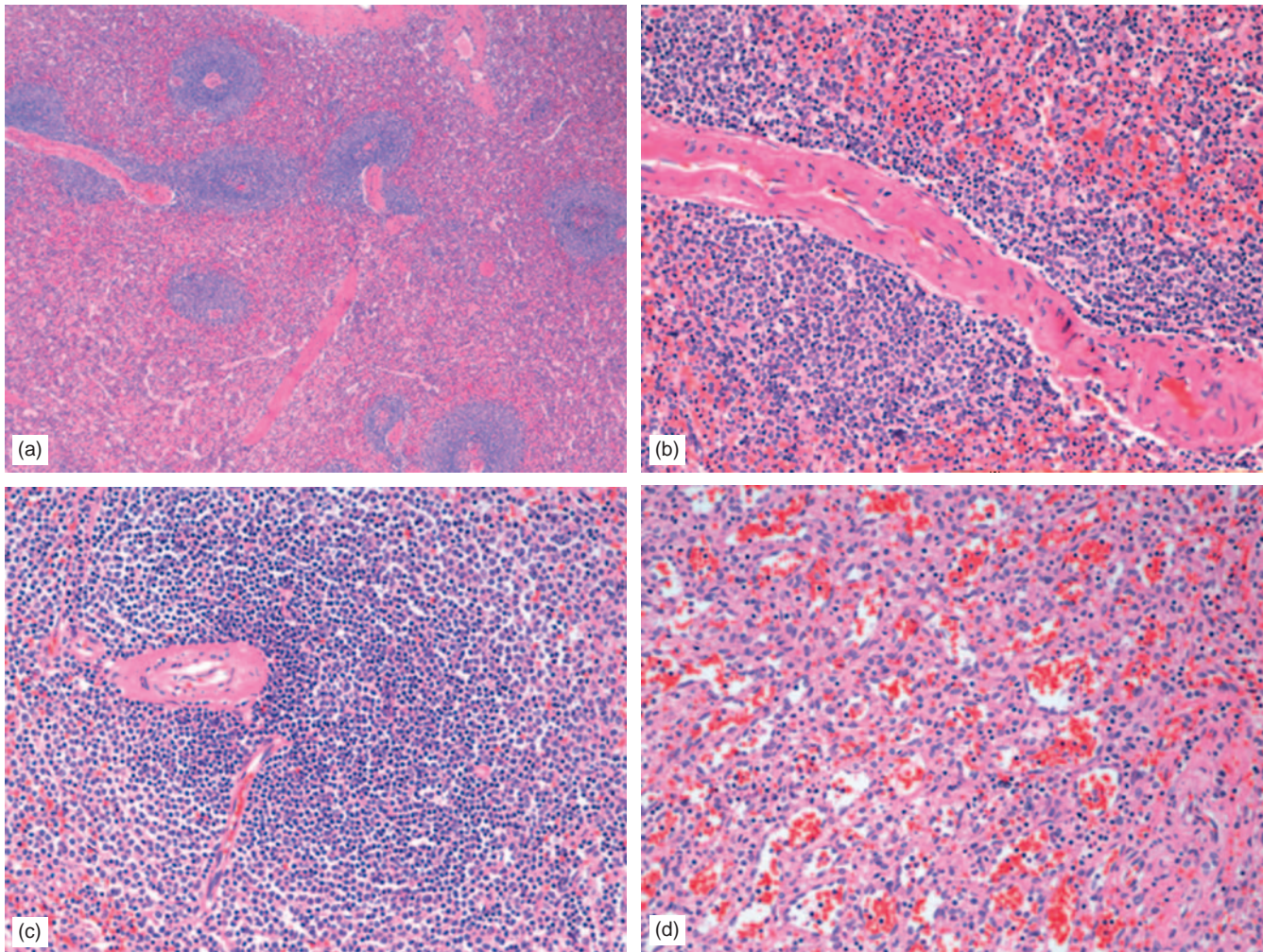


FIGURE 1.31 (a) A low power microscopic view of the spleen demonstrated white and red pulp. (b) and (c) show higher power views of the T- and B-cell regions, respectively. (d) A higher power view of the red pulp with numerous blood-containing sinuses.

into the white pulp initiates an adaptive immune response against the blood-borne pathogens.

The Red Pulp

The red pulp consists of splenic cords and the sinusoidal system (Figures 1.30 and 1.31) [116–118,121]. Cords are composed of a meshwork of fibroblast-like cells supported by extracellular matrix and reticulin fibers. They form cavernous spaces with no endothelial lining and directly receive arterial blood from terminal arterioles and arterial capillaries. Numerous macrophages are present in the cords which are able to remove the damaged, abnormal, or aged blood cells, while the blood passes through into the venous sinuses. Unlike the cords, these sinuses are lined by the endothelial cells. There are slit-like gaps between the endothelial cells which allow blood cells to penetrate from the cordal space into the sinusoidal lumen. Abnormal RBCs, such as sickle cells, or cells with inclusions, such as Heinz bodies, might not be able to pass through these

slits. The sinus basement membrane consists of a network of contractile thick and thin reticular fibers (stress fibers) running circumferentially and longitudinally, respectively. The network is connected to the extracellular matrix of the splenic cord and its contraction helps the blood to pass through the cords into the sinuses. Activity of stress fibers might also help to retain erythrocytes and platelets in the spleen, thereby forming a reservoir for these cells.

The spleen demonstrates three major functions:

1. **Phagocytosis:** Invading micro-organisms and pathogens are effectively removed by macrophages in the spleen. Also, abnormal, damaged, and dysfunctional blood cells are filtered and removed by macrophages when blood passes through the spleen.
2. The splenic white pulp is an important component of the cell-mediated and humoral immune systems.
3. The splenic sinus system serves as a big reservoir for blood cells.

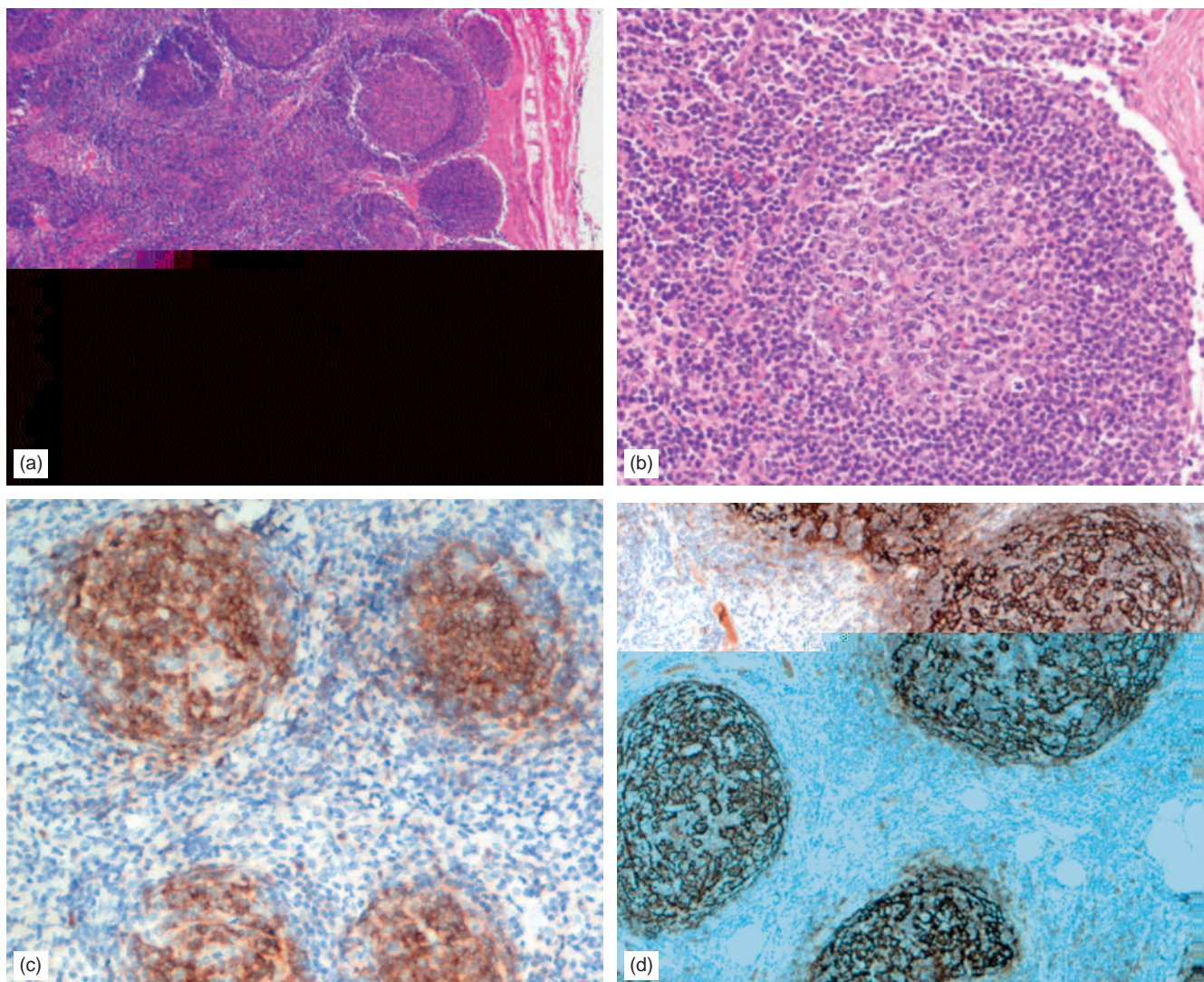


FIGURE 1.32 (a) An H&E section of a lymph node demonstrating cortex and paracortex, primary and secondary follicles, and medulla. (b) A higher power view demonstrating a secondary follicle with a germinal center surrounded by a mantle zone, (c) demonstrating CD10 expression on follicular B-cells, and (d) showing CD21 expression on follicular dendritic cells.

STRUCTURE AND FUNCTION OF THE LYMPH NODES

Lymph nodes are the major components of the lymphatic system and consist of round or oval structures located along the major blood vessels, in peritoneum and mediastinum, and at the base of the extremities. They measure from several millimeters to around 1 cm in diameter and are surrounded by a fibrous capsule. Incoming lymphatic vessels penetrate the capsule and release their content into the subcapsular sinuses. Blood vessels enter and leave the lymph nodes through the hilum. Several fibrous trabeculae extend from the inner part of the capsule into the lymph node parenchyma, forming a supporting meshwork and dividing the lymph node into many subsections. The lymph node

parenchyma is divided into a peripheral zone, the *cortex*, and a deeper, centrally located zone, the *medulla* (Figure 1.32) [122–124]. The cortex consists of a superficial part, immediately located under the capsule, and a deeper part or *paracortex*. The following anatomical structures are recognized in lymph node sections (Figure 1.32).

Follicular Structures

Follicular structures are the primary home of the B lymphocytes [122, 124–127]. The ones that are not yet exposed to antigens are called primary follicles and consist of packed, uniform-looking small mature lymphocytes. Secondary follicles have been already exposed to antigenic stimulation. They have a pale central area, called *germinal*

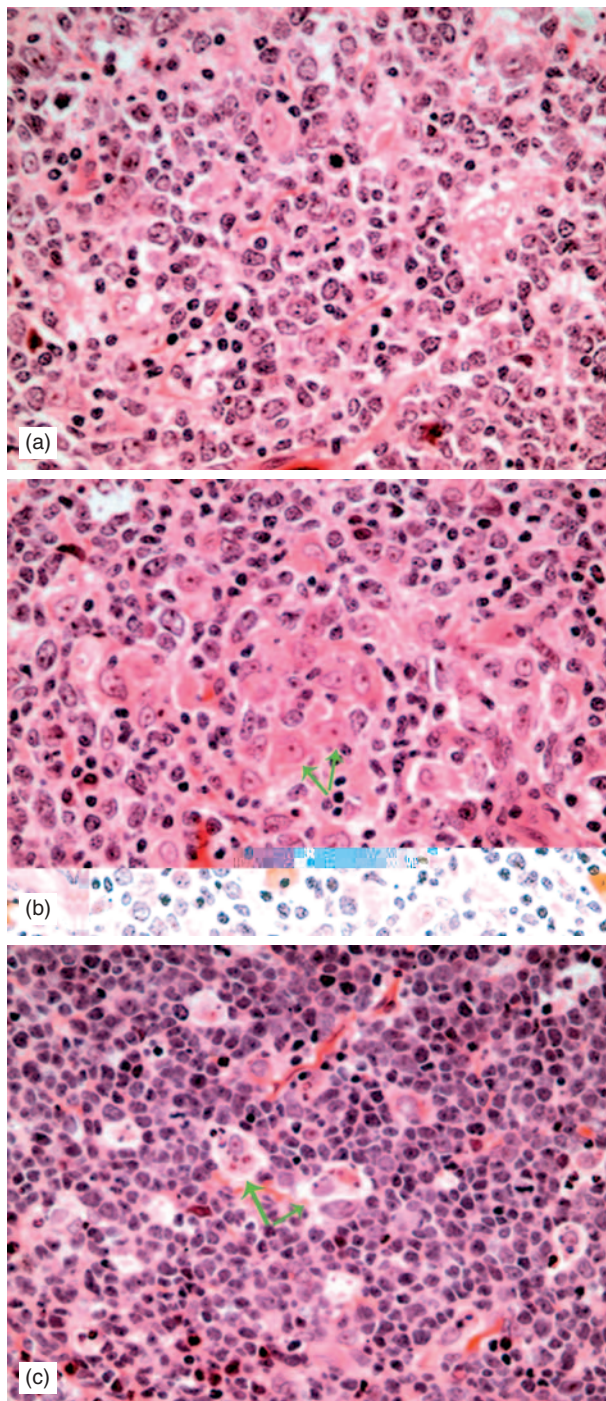


FIGURE 1.33 High power views of a germinal center in a lymph node section demonstrating centroblasts and centrocytes with the presence of mitotic figures (a, b, and c), follicular dendritic cells (b, arrows), and tingible body macrophages (c, arrows).

center, consisting of a mixture of large and small cells. The smaller uniform-looking lymphocytes surrounding the germinal center are packed as a darkly stained crescent known as *mantle zone* (Figures 1.32 and 1.33). Lymphocytes of the mantle zones (mantle cells) are B-cells, but functionally heterogeneous, consisting of bone marrow-derived naive cells

and lymphocytes previously exposed to antigens. However, a significant proportion of the mantle cells express B-cell associated markers, such as CD19, CD20, CD79a, as well as CD5, FMC-7, and CD43. Mantle cells are negative for CD10 and CD23.

Within the germinal center, two types of lymphoid cells are present [124, 126, 127]:

1. *Centroblasts*, which are apparently derived from mantle cells and are large, non-cleaved cells located at the bottom of the germinal center and often show frequent mitotic figures. They have a vesicular nuclear chromatin, and multiple distinct nucleoli usually located close to the nuclear membrane (Figure 1.33).
2. *Centrocytes*, which are evolved from centroblasts and consist of small cleaved lymphoid cells located at the upper part of the germinal centers. These cells show scant cytoplasm, dispersed nuclear chromatin, and inconspicuous nucleoli. Centrocytes mature to marginal zone or monocytoid B-cells and leave the germinal center (Figure 1.33).

Centroblasts and centrocytes express B-cell-associated antigen, such as CD19, CD20, CD22, and CD79a. They are often positive for CD10 and bcl-6 and negative for CD5.

In addition to the B lymphocytes, follicles contain FDCs, which function as antigen presenting cells (Figure 1.32d) [124, 126, 127]. FDCs are derived from the mesenchymal cells in the follicular structures (not originated from bone marrow stem cells). They often appear in pairs, have round or irregular nuclei with dispersed chromatin, and often one small, centrally located nucleolus. FDCs are characterized by expressing CD21 and CD35. Also, scattered macrophages, some with tingible bodies, are present in the germinal centers (Figure 1.32c).

The Paracortex

The paracortical area is the primary home of T-cells. These cells slowly flow in the spaces provided by the paracortical cords. The cords consist of a centrally located venule lined by tall, cuboidal endothelial cells (high endothelial venules) surrounded by narrow corridors outlined by reticular fibers [124, 127, 128]. In these corridors T-cells interact with antigens presented to them by the stationary IDCs. IDCs, unlike FDCs, are derived from bone marrow stem cells and express HLA-DR and S-100 protein. The T-cells have passed through the thymic developmental processes (post-thymic T-cells) and are divided into *helper* and *suppressor* T-cells. Helper T-cells are CD4-positive and release regulatory cytokines to facilitate the immune responses and are divided into two major subtypes: (1) Th₁ cells which secrete IL-2 and interferon γ and provide help to other T-cells and macrophages and (2) Th₂ cells which secrete IL-4, IL-5, IL-6, and IL-10 and assist B-cells in their antibody production. Suppressor T-cells express CD8 and are primarily involved in cytotoxic reactions. There are more CD4-positive than CD8-positive T-cells in lymph nodes.

The Medulla

The medulla consists of medullary cords loaded with T- and B-cells, plasma cells, and macrophages [126, 127].

Vascular and Lymphatic Structures

The main artery, after entering the lymph node through the hilum, branches and gives rise to numerous arterioles that pass through the trabeculae and reach the cortex [126, 127, 129]. There, they make a capillary network. The capillaries empty into the high endothelial venules in the center of the cortical corridors. Venules join together and make larger branches, extend from the cortex to the medulla, and finally leave the hilum as veins.

Afferent lymphatic vessels penetrate the lymph node capsule and empty into the subcapsular sinuses, which are connected to the cortical sinuses. The sinuses are lined by endothelial cells which have no basement membrane. The sinusoidal lumen is subdivided into smaller interconnecting spaces by fibrous septa covered by endothelial cells. Sinuses guide the lymphatic flow from the capsule into the medulla and eventually terminate to the efferent lymphatics at the hilum and leave the lymph node. Sinusoidal spaces are loaded with macrophages.

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Principles of Immunophenotyping

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THE HUMAN CELL DIFFERENTIATION MOLECULES

“Human cell differentiation molecules”(HCDM) is a new terminology coined by the 8th International Workshop on Human Leukocyte Differentiation Antigen (HLDA) to describe surface molecules associated with human cell differentiation [1–3]. These molecules have been characterized in a series of international workshops studying a large number of monoclonal antibodies. The antibodies have been grouped according to their patterns of reactivity and are referred to as “clusters of differentiation”(CD). The 8th International Workshop on HLDA, held in Adelaide, Australia, December 2004, brought the total number of CD molecules to 339 (Table 2.1) [1–3]. These molecules characterize human leukocytes as well as other human cells such as endothelial and stromal cells. They are not only detected on the surface but also inside the cells [1–3].

Monoclonal antibodies are routinely used for the diagnosis and classification of hematopoietic malignancies and other hematologic disorders. However, it is important to remember the following facts:

1. If not all, by far, the vast majority of the available monoclonal antibodies raised against CD molecules are not tumor-specific and react with non-neoplastic hematopoietic cells.
2. These molecules are mostly differentiation associated and not lineage-specific.
3. They may react with non-hematopoietic human cells.

Because of these facts, the results of immunophenotypic studies, such as flow cytometry and immunohistochemical stains, should be

always incorporated with morphology and other available data, such as cytogenetics and molecular studies. The following are examples of CD molecules most frequently used in diagnostic hematopathology at the present time [4–7].

B-CELL-ASSOCIATED CD MOLECULES

CD10

CD10, also known as *common acute lymphoblastic leukemia antigen* (CALLA), is a neutral endopeptidase, which cleaves peptides at the amino side of hydrophobic residues and inactivates several peptide hormones [5, 8]. It is expressed on the leukemic cells of the most common type of acute lymphoblastic leukemia (ALL), precursor B-cell ALL. CD10 is also present in hematogones (normal precursor B-cells in bone marrow), as well as cells in other B-cell lymphoid malignancies, such as follicular center cell lymphomas, Burkitt leukemia/lymphoma, and some cases of plasma cell myeloma, T-cell ALL, and acute myelogenous leukemia (AML) [4–6, 8]. This molecule is abundant in kidney, particularly on the brush border of proximal tubules and on glomerular epithelium [9, 10]. It is also present in granulocytes, fibroblasts, and a variety of normal and neoplastic epithelial cells [5, 9].

CD19

CD19 is a signal-transduction molecule that plays an important role in the regulation of development, activation, and differentiation of B-lymphocytes [5, 11]. It is the earliest lineage-restricted molecule expressed on B-cells throughout B-cell differentiation. Follicular dendritic cells also express

TABLE 2.1 The human cell differentiation molecules.

CD	Molecule	Main distribution
CD1a	T6/Leu-6, R4, HTA1	Cortical thymocyte, LC, IDC
CD1b	R1	Cortical thymocyte, LC, IDC
CD1c	M241, R7	Cortical thymocyte, LC, IDC
CD2	T11; Tp50; sheep red blood cell (SRBC) receptor; LFA-2	Thymocyte, T, NK, thymic B-cells
CD3	CD3 complex, T3, Leu4	Precursor T, thymocyte, T
CD4	OKT4, Leu 3a, T4	Helper T, thymocyte, M
CD5	Tp67; T1, Ly1, Leu-1	Thymocytes, T, B subset
CD6	T12	Thymocytes, T, B subset
CD7	Leu 9, 3A1, gp40, T-cell leukemia antigen	Precursor T, T, NK
CD8	OKT8, LeuT, LyT2, T8	Cytotoxic T, NK
CD9	Drap-27, MRP-1, p24, leukocyte antigen MIC3	Platelet, early B, Eo, Baso, endothelial
CD10	CALLA, membrane metallo-endopeptidase	Precursor B, B subset, G
CD11a	alphaL; LFA-1, gp180/95	All leukocytes
CD11b	alphaM; α -chain of C3bi receptor, gp155/95, Mac-1, Mo1	G, M, NK
CD11c	alphaX; α -chain of: complement receptor type 4 (CR4); gp150/95	G, M, NK
CDw12	P90-120	G, M, NK
CD13	Aminopeptidase N, APN, gp150, EC 3.4.11.2	G, M, endothelial, LGL subset
CD14	LPS receptor	M, DC subset
CD15	Lewis X, CD 15u: sulphated Lewis X. CD 15s: sialyl Lewis X	G, Reed-Sternberg cells
CD16	Fc gamma R IIIa	NK, G, M, macrophage
CDw17	LacCer, lactosylceramide	Platelet, G, M, B subset
CD18	β 2-Integrin chain, macrophage antigen 1 (mac-1)	All leukocytes
CD19	Bgp95, B4	Precursor B, B
CD20	B1; membrane-spanning 4-domains, subfamily A, member 1	Precursor B subset, B
CD21	C3d receptor, CR2, gp140; EBV receptor	FDC, B subset, T subset
CD22	Bgp135; BL-CAM, Siglec2	Precursor B, B
CD23	Low affinity IgE receptor; Fc ϵ RII; gp50-45; Blast-2	B, DC, M
CD24	Heat stable antigen homologue (HSA), BA-1	Precursor B, B, G
CD25	Interleukin (IL)-2 receptor α -chain; Tac-antigen	Activated T, B and M
CD26	Dipeptidylpeptidase IV; gp120; Ta1	Thymocyte, B, NK, macrophage, activated T
CD27	T14, S152	NK, thymocyte, B subset, T subset
CD28	Tp44	Thymocyte, T, PC
CD29	Integrin β 1 chain; platelet GPIIa; VLA (CD49) β -chain	All leukocytes
CD30	Ki-1 antigen, Ber-H2 antigen	M, activated B, T, and NK
CD31	PECAM-1; platelet GPIIa'; endocam	Endothelial, platelet, leukocyte

(Continued)

TABLE 2.1 (Continued)

CD	Molecule	Main distribution
CD32	Fc gamma receptor type II (FcγRII), gp40	M, G, Eo, Baso, B, platelet
CD33	My9, gp67, p67	Precursor G, G, M
CD34	My10, gp105-120	Hematopoietic progenitor cells, endothelium
CD35	C3b/C4b receptor; complement receptor type 1 (CR1)	Erythroid, B, Eo, M, T subset
CD36	Platelet GPIV, GPIIb, OKM-5 antigen	Platelet, M
CD37	Gp40-52	Mature B
CD38	T10; gp45, ADP-ribosyl cyclase	Early and activated hematopoietic cells, PC
CD39	Gp80, ectonucleoside triphosphate diphosphohydrolase 1	Leukocytes
CD40	Bp50, TNF Receptor 5	B, DC, macrophage, endothelial
CD41	Platelet glycoprotein GPIIb	Platelet
CD42a	Platelet glycoprotein GPIX	Platelet
CD42b	Platelet glycoprotein GPIb-a	Platelet
CD42c	Platelet glycoprotein GPIb-β	Platelet
CD42d	Platelet glycoprotein GPV	Platelet
CD43	Leukosialin; gp95; sialophorin; leukocyte sialoglycoprotein	Leukocytes
CD44	Pgp-1; gp80-95, Hermes antigen, ECMR-III and HUTCH-I.	Leukocytes
CD45	LCA, B220, protein tyrosine phosphatase, receptor type, C	Leukocytes
CD45RA	Restricted T200; gp220; isoform of leukocyte common antigen	Naive T, B, M, NK
CD45RO	Restricted T200; gp180	Thymocyte, memory T, G, M
CD45RB	Restricted T200; isoform of leukocyte common antigen	T subset, B, G, M
CD46	Membrane cofactor protein (MCP)	Leukocytes
CD47	Integrin-associated protein (IAP), Ovarian carcinoma antigen OA3	Leukocytes
CD48	BLAST-1, Hulym3, OX45, BCM1	Leukocytes
CD49a	Integrin α1 chain, very late antigen, VLA 1a	Broad
CD49b	Integrin α2 chain, VLA-2-α chain, platelet gplα	Broad
CD49c	Integrin α3 chain, VLA-3-α chain	Broad
CD49d	Integrin α4 chain, VLA-4-α chain	Broad
CD49e	Integrin α5 chain, VLA-5-α chain	Broad
CD49f	Integrin α6 chain, VLA-6-α chain, platelet gplc	Broad
CD50	ICAM-3, intercellular adhesion molecule 3	Leukocytes
CD51	Integrin α chain, vitronectin receptor α chain	Platelet, endothelial cell
CD52	Campath-1, HE5	Thymocyte, B, T, NK, M
CD53	MRC OX-44	B, T, M, NK, G
CD54	ICAM-1, intercellular adhesion molecule 1	B, T, M, G, endothelial cell
CD55	DAF, decay accelerating factor	Broad

(Continued)

TABLE 2.1 (Continued)

CD	Molecule	Main distribution
CD56	NKHI, neural cell adhesion molecule (NCAM)	NK, T subset, neuroendodermal cells
CD57	HNK1	NK, T subset, neuroendodermal cells
CD58	LFA-3, lymphocyte function associated antigen-3	Broad
CD59	MACIF, MIRL, P-18, protectin	Broad
CD60	GD3 (CD60a), 9-0-acetyl GD3 (CD60b), 7-0-acetyl GD3 (CD60c)	Platelets, T subset
CD61	Glycoprotein IIIa, beta3 integrin	Platelets
CD62E	E-selectin, LECAM-2, ELAM-1	Endothelium
CD62L	L-selectin, LAM-1, Mel-14	B, T, M, NK subset, G
CD62P	P-selectin, granule membrane protein-140 (GMP-140)	Activated platelet, endothelium
CD63	LIMP, gp55, LAMP-3 neuroglandular antigen, granulophysin	Activated platelets, G, M, endothelium
CD64	FcγR1, FcγR1	Precursor G, G, M, DC subset
CD65	Ceramide dodecasaccharide 4c, VIM2	G, M
CD66a	BGP, carcinoembryonic antigen-related cell adhesion molecule 1	G, epithelium
CD66b	CGM6, NCA-95	G
CD66c	Nonspecific cross-reaction antigen, NCA-50/90	G, epithelium
CD66d	CGM1	G
CD66e	CEA	Epithelium
CD66f	PSG, Sp-1, pregnancy specific (b1) glycoprotein	Myeloid cell lines, placenta
CD68	gp110, macrosialin	M, G, DC subset, Baso, Mast cell
CD69	AIM, activation inducer molecule, MLR3, EA1, VEA	Activated leukocytes
CD70	CD27 ligand, KI-24 antigen	Activated B and T
CD71	Transferrin receptor	Erythroid precursors, proliferating cells
CD72	Lyb-2, Ly-19.2, Ly32.2	Precursor B, B
CD73	Ecto-5'-nucleotidase	B subset, T subset
CD74	MHC Class II associated invariant chain (Ii)	B, IDC, T subset
CD75	Lactosamines	B, activated T, macrophages, activated endothelium
CD75s	Since HLDA7, CDw76 has been renamed CD75s	B, T subset
CD77	Pk blood group antigen; Burkitt's lymphoma associated antigen	Germinal center B
CD79a	MB-1; Igα	Precursor B, B, activated B
CD79b	B29; Igβ	Precursor B, B, activated B
CD80	B7-1; BB1	Macrophages, activated T and B
CD81	Target of an antiproliferative antibody (TAPA-1); M38	Broad
CD82	R2; 4F9; C33; IA4, kangai 1	Broad
CD83	HB15	IDC, LC

(Continued)

TABLE 2.1 (Continued)

CD	Molecule	Main distribution
CD84	p75, GR6	CD84
CD85	ILT5; LIR3; HL9	B, thymocytes, M, macrophages, platelets
CD86	B7-2; B70	IDC, LC, B, and M subset
CD87	Urokinase plasminogen activator-receptor (uPA-R)	Subsets of T, NK, M and G
CD88	C5a-receptor	G, M, DC
CD89	Fca-receptor, IgA-receptor	Precursor myeloid, G, M
CD90	Thy-1	Hematopoietic stem cell
CD91	α 2-macroglobulin receptor (ALPHA2M)	Broad
CDw92	p70	G, M
CDw93	GR11	G, M, myeloid blast, endothelium
CD94	kP43, killer cell lectin-like receptor subfamily D, member 1	NK, T subset
CD95	APO-1, Fas, TNFRSF6	Thymocytes, B and T subset
CD96	TACTILE (T-cell activation increased late expression)	Activated NK and T
CD97	BL-KDD/F12	DC, G, M activated B and T
CD98	4F2, FRP-1	Activated leukocytes
CD99	MIC2, E2	Broad
CD100	SEMA4D	Leukocytes, activated T, germinal center B
CD101	V7, P126	G, M, DC, activated T
CD102	ICAM-2	M, platelet, endothelium
CD103	Integrin alpha E subunit, HML-1	Intraepithelial lymphocytes, hairy cells
CD104	Integrin beta 4 subunit, TSP-1180	Epithelium
CD105	Endoglin	Endothelium, precursor B, activated M
CD106	VCAM-1 (vascular cell adhesion molecule-1), INCAM-110	DC, activated endothelium
CD107a	Lysosomal associated membrane protein (LAMP)-1	Degranulated platelet, activated T
CD107b	Lysosomal associated membrane protein (LAMP)-2	Degranulated platelet
CD108	GPI-gp80; John-Milton-Hagen (JMH) human blood group antigen	Erythroid
CD109	Platelet activation factor; 8A3, E123	Activated platelet, endothelium
CD110	Thrombopoietin receptor; c-mpl	Hematopoietic stem cells, platelets
CD111	PRR1, Nectin 1, Hve C1, poliovirus receptor related 1 protein	34 ⁺ hematopoietic precursors
CD112	PRR2, Nectin 2, Hve B, poliovirus receptor related 2 protein	34 ⁺ hematopoietic precursors
CDw113	PVRL3, Nectin3	Epithelium
CD114	G-CSFR, HG-CSFR, CSFR3	M, platelets
CD115	M-CSFR, CSF-1, C-fms	M, macrophages
CD116	GM-CSF R alpha subunit	Myeloid cells

(Continued)

TABLE 2.1 (Continued)

CD	Molecule	Main distribution
CD117	SCFR, c-kit, stem cell factor receptor	Hematopoietic stem cells, mast cells, plasma cells, AML blasts
CD118	LIFR	Broad
CD119	IFN gamma receptor alpha chain	Broad
CD120a	TNFR1; TNFRp55	Broad
CD120b	TNFR2; TNFRp75	Broad
CD121a	Type I IL-1 receptor	Broad
CD121b	Type II IL-1 receptor	Broad
CD122	IL-2 receptor betachain, p75	B, T, NK, M
CD123	Interleukin-3 receptor alpha chain (IL-3Ra)	Hematopoietic precursors
CD124	IL-4 R alpha chain	Broad
CDw125	Interleukin-5 receptor alpha chain	Baso, Eo, activated B
CD126	IL-6 receptor alpha chain	T, M, activated B
CD127	IL-7 receptor alpha chain, p90	Precursor B, B, T
CD129	IL-9 receptor alpha chain	Hematopoietic cells
CD130	gp130	Broad
CD131	Common β chain, low-affinity (granulocyte-macrophage)	Precursor myeloid, precursor B, M, G, Eo
CD132	Common gamma chain, interleukin 2 receptor, gamma	B, T, M, G, NK
CD133	AC133, PROM1, prominin 1	CD34 ⁺ hematopoietic precursor
CD134	OX 40, TNFRSF4	Thymocyte, T
CD135	FLT3, STK-1, flk-2	Precursor B, precursor myelomonocytic
CDw136	Macrophage stimulating protein receptor, MSP-R, RON	Epithelium, M
CDw137	4-1BB, Induced by lymphocyte activation (ILA)	T, activated T
CD138	Syndecan-1, B-B4	Plasma cells, B subset, epithelium
CD139		B, M, G
CD140a	Alpha-platelet derived growth factor (PDGF) receptor	Mesenchymal cells
CD140b	Beta-platelet derived growth factor (PDGF) receptor	Mesenchymal cells, M, G
CD141	Thrombomodulin (TM), fetomodulin	Broad
CD142	Tissue factor, thromboplastin, coagulation factor III	Epithelium, M, endothelium
CD143	Angiotensin-converting enzyme (ACE), peptidyl dipeptidase A	Broad
CD144	VE-cadherin, cadherin-5	Endothelium
CDw145	None	Endothelium
CD146	Muc 18, MCAM, Mel-CAM, s-endo	Endothelium, melanoma cells, activated T
CD147	Basigin, M6, extracellular metalloproteinase inducer (EMMPRIN)	Leukocyte, erythroid, platelet, endothelium
CD148	DEP-1, HPTP-n, protein tyrosine phosphatase, receptor type, J	G, M, T subset, DC, platelet

(Continued)

TABLE 2.1 (Continued)

CD	Molecule	Main distribution
CD150	SLAM, signalling lymphocyte activation molecule, IPO-3	Thymocyte, B, DC, T subset, endothelium
CD151	Platelet-endothelial tetra-span antigen (PETA)-3	Platelet, endothelium, epithelium
CD152	Cytotoxic T-lymphocyte antigen (CTLA)-4	Activated B and T
CD153	CD30 Ligand	Activated T and M
CD154	CD40 Ligand; TRAP (TNF-related activation protein)-1; T-BAM	Activated T
CD155	Polio virus receptor (PVR)	M, neurons
CD156a	ADAM-8, a disintegrin and metalloproteinase domain 8	G, M
CD156b	TACE, ADAM 17 snake venom like protease CSVp	Broad
CD157	BST-1 BP-3/IF7 Mo5	G, M, precursor B
CD158	Killer cell Ig-like receptor, three domains, long cytoplasmic tail, 1	NK, T subset
CD159a	Killer cell lectin-like receptor subfamily C, member 1	NK
CD160	BY55, NK1, NK28	NK, T subset
CD161	NKR-P1A, killer cell lectin-like receptor subfamily B, member 1	NK, T subset
CD162	P selectin glycoprotein ligand 1, PSGL-1	T, M, G, B subset
CD163	GHI/61, D11, RM3/1, M130	M, macrophage, activated T
CD164	MUC-24, MGC 24, multi-glycosylated core protein 24	M, epithelium, bone marrow stromal cells
CD165	AD2, gp 37	Thymocyte, T, platelet
CD166	ALCAM, KG-CAM, activated leukocyte cell adhesion molecule	Epithelium, activated T and M
CD167	Discoidin receptor DDR1 (CD 167a) and DDR2 (CD 167b)	Epithelium
CD168	RHAMM (receptor for hyaluronan involved in migration and motility)	Thymocyte
CD169	Sialodhesin, Siglec-1	Macrophage
CD170	Siglec 5 (sialic acid binding Ig-like lectin 5)	Myeloid cells
CD171	Neuronal adhesion molecule, LI	Neurons
CD172	SIRP, signal inhibitory regulatory protein family member	Leukocytes
CD173	Blood Group H2	Erythrocytes
CD174	Lewis Y blood group, LeY, fucosyltransferase 3	Erythrocytes
CD175	Tn Antigen (T-antigen novelle)	Carcinomas
CD176	Thomsen-Friedenreich (TF) antigen	Carcinomas
CD177	NB 1	
CD178	FAS ligand, CD95 ligand	T, NK
CD179a	V pre beta	Precursor B
CD179b	Lambda 5	Precursor B
CD180	RP105, Bgp95	Mantle zone and marginal zone B
CD181	CXCR1 (was CDw128A)	Leukocytes
CD182	CXCR2 (was CDw128B)	Leukocytes

(Continued)

TABLE 2.1 (Continued)

CD	Molecule	Main distribution
CD183	CXCR3 chemokine receptor, G protein-coupled receptor 9	T, CD34 ⁺ hematopoietic cells, DC subset, Eo
CD184	CXCR4 chemokine receptor, Fusin	M, T subset
CD185	CXCR5; Chemokine (C-X-C motif) Receptor 5, Burkitt lymphoma receptor 1	Broad
CDw186	CXCR6; Chemokine (C-X-C motif) Receptor 6	T, epithelium
CD191	CCR1; Chemokine (C-C motif) Receptor 1, RANTES Receptor	T and NK subset
CD192	CCR2; Chemokine (C-C motif) Receptor 2, MCP-1 receptor	M
CD193	CCR3; Chemokine (C-C motif) Receptor 3, eosinophil eotaxin receptor	Eo, Baso, epithelium
CD195	CCR5; Chemokine receptor	T, M
CD196	CCR6; Chemokine (C-C motif) Receptor 6	DC and T subset
CD197	CCR7; (was CDw197) Chemokine (C-C motif) Receptor 7	DC and T subset
CDw198	CCR8; Chemokine (C-C motif) Receptor 8	Thymocyte, macrophage
CDw199	CCR9; Chemokine (C-C motif) Receptor 9	Intestinal T-cells
CD200	MRC OX 2	Broad
CD201	Endothelial protein C receptor (EPCR)	Endothelium
CD202b	TIE2, TEK	Endothelium, hematopoietic stem cell
CD203c	E-NPP3, PDNP3, PD-1beta	Mast cell, Baso
CD204	MSR, SRA, Macrophage scavenger receptor	Macrophage
CD205	DEC-205	DC
CD206	Macrophage mannose receptor (MMR)	M, macrophage, endothelium
CD207	Langerin	LC
CD208	DC-LAMP	IDC
CD209	DC-SIGN	DC subset
CDw210	IL-10 receptor	B, T, NK, M, macrophage
CD212	IL-12 receptor beta chain	Activated T and NK
CD213a1	IL-13 receptor alpha 1	Broad
CD213a2	IL-13 R alpha 2	B, M
CDw217	IL-17 receptor	Broad
CDw218	IL18Ralpha	
CD220	Insulin receptor	Broad
CD221	IGF I Receptor, type I IGF receptor	Broad
CD222	Mannose-6-phosphate receptor, insulin like growth factor II R	Broad
CD223	LAG-3 (Lymphocyte activation gene 3)	T and NK subset
CD224	Gamma-glutamyl transferase, GGT	Broad
CD225	Leu-13, interferon-induced transmembrane protein 1	Broad
CD226	DNAM-1, DTA-1	T, NK, M, platelet, B subset

(Continued)

TABLE 2.1 (Continued)

CD	Molecule	Main distribution
CD227	MUC1; episialin; PUM; PEM; EMA; DF3 antigen; H23 antigen	Broad
CD228	Melanotransferrin, p97	Melanoma cells, endothelium
CD229	Ly9	T, B
CD230	Prion protein, PrP(c), PrP(sc) abnormal form	Broad
CD231	TALLA-1, TM4SF2	Precursor T, neuroblastoma
CD232	VESPR	Broad
CD233	Band 3, AE1, anion exchanger 1, Diego blood group antigen	RBC
CD234	DARC, Fy-glycoprotein, Duffy blood group antigen	RBC
CD235a	Glycophorin A	RBC
CD235b	Glycophorin B	RBC
CD236	Glycophorin C/D	RBC, stem cell subset
CD236R	Glycophorin C	RBC, stem cell subset
CD238	Kell blood group antigen	RBC, stem cell subset
CD239	B-CAM, lutheran glycoprotein	RBC, stem cell subset
CD240CE	Rh blood group system, Rh30CE	RBC
CD240D	Rh blood group system, Rh30D	RBC
CD240DCE	Rh30D/CE crossreactive mabs	RBC
CD241	RhAg, Rh50, Rh associated antigen	RBC
CD242	LW blood group, Landsteiner-Wiener blood group antigens	RBC
CD243	MDR-1, P-glycoprotein, pgp 170, multidrug resistance protein I	Hematopoietic stem cell
CD244	2B4; NAIL; p38	NK, T subset
CD245	p220/240, DY12, DY35	T subset
CD246	Anaplastic lymphoma kinase (ALK)	Anaplastic large cell lymphoma
CD247	T-cell receptor zeta chain, CD3 zeta	T, NK
CD248	TEM1, Endosialin	Fibroblast, endothelium
CD249	Aminopeptidase A; APA, gp160	Epithelium
CD252	OX40L; TNF (ligand) superfamily member 4, CD134 ligand	T
CD253	TRAIL; TNF (ligand) superfamily member 10, APO2L	T
CD254	TRANCE; TNF (ligand) superfamily member 11, RANKL	T, M
CD256	APRIL; TNF (ligand) superfamily member 13, TALL2	Osteoclast, B subset
CD257	BLYS; TNF (ligand) superfamily, member 13b, TALL1, BAFF	B
CD258	LIGHT; TNF (ligand) superfamily, member 14	
CD261	TRAIL-R1; TNFR superfamily, member 10a, DR4, APO2	Broad
CD262	TRAIL-R2; TNFR superfamily, member 10b, DR5	Broad
CD263	TRAIL-R3; TNFR superfamily, member 10c, DCR1	Broad

(Continued)

TABLE 2.1 (Continued)

CD	Molecule	Main distribution
CD264	TRAIL-R4; TNFR superfamily, member 10d, DCR2	NK, T subset
CD265	TRANCE-R; TNFR superfamily, member 11a, RANK	M, DC
CD266	TWEAK-R; TNFR superfamily, member 12A, type I transmembrane protein Fn14	Broad
CD267	TACI; TNFR superfamily, member 13B, transmembrane activator and CAML interactor	Lymphocytes
CD268	BAFFR; TNFR superfamily, member 13C, B-cell-activating factor	B
CD269	BCMA; TNFR superfamily, member 17, B-cell maturation factor	B
CD271	NGFR (p75); nerve growth factor receptor (TNFR superfamily, member	Neurons
CD272	BTLA; B and T-lymphocyte attenuator	B, T subset
CD273	B7DC, PDL2; programmed cell death 1 ligand 2	Activated B and T
CD274	B7H1, PDL1; programmed cell death 1 ligand 1	Broad
CD275	B7H2, ICOSL; inducible T-cell co-stimulator ligand (ICOSL)	Broad
CD276	B7H3; B7 homolog 3	N/A
CD277	BT3.1; B7 family: butyrophilin, subfamily 3, member A1	
CD278	ICOS; inducible T-cell co-stimulator	Activated T
CD279	PD1; programmed cell death 1	Broad
CD280	ENDO180; uPARAP, mannose receptor, C type 2, TEM22	Macrophages
CD281	TLR1; TOLL-like receptor 1	Lymphocytes
CD282	TLR2; TOLL-like receptor 2	Lymphocytes
CD283	TLR3; TOLL-like receptor 3	Lymphocytes
CD284	TLR4; TOLL-like receptor 4	Lymphocytes
CD289	TLR9; TOLL-like receptor 9	Lymphocytes
CD292	BMPRI1A; bone morphogenetic protein receptor, type IA	Broad
CDw293	BMPRI1B; bone morphogenetic protein receptor, type IB	Broad
CD294	CRTH2; PGRD2; G protein-coupled receptor 44,	T subset
CD295	LEPR; leptin receptor	Platelets, G
CD296	ART1; ADP-ribosyltransferase 1	G
CD297	ART4; ADP-ribosyltransferase 4; Dombrock blood group glycoprotein	RBC
CD298	ATP1B3; Na ⁺ /K ⁺ -ATPase beta 3 subunit	Broad
CD299	DCSIGN-related; CD209 antigen-like, DC-SIGN2, L-SIGN	DC
CD300	CMRF35 FAMILY; CMRF-35H	M, G, B and T subsets
CD301	MGL; CLECSF14, macrophage galactose-type C-type lectin	Macrophages
CD302	DCL1; Type I transmembrane C-type lectin receptor DCL-1	Hodgkin lymphoma cell line
CD303	BDCA2; C-type lectin, superfamily member 11	DC subtype
CD304	BDCA4; Neuropilin 1	Broad
CD305	LAIR1; leukocyte-associated Ig-like receptor 1	B, T, NK

(Continued)

TABLE 2.1 (Continued)

CD	Molecule	Main distribution
CD306	LAIR2; leukocyte-associated Ig-like receptor 2	B, T, NK
CD307	IRTA2; immunoglobulin superfamily receptor translocation associated	B
CD309	VEGFR2; KDR (a type III receptor tyrosine kinase)	Endothelium
CD312	EMR2; EGF-like module containing, mucin-like, hormone receptor-like	Lymphocytes
CD314	NKG2D; killer cell lectin-like receptor subfamily K, member 1	NK
CD315	CD9P1; prostaglandin F2 receptor negative regulator	Lymphocytes
CD316	EWI2; immunoglobulin superfamily, member 8	Lymphocytes
CD317	BST2; bone marrow stromal cell antigen 2	Bone marrow stromal cells
CD318	CDCP1; CUB domain-containing protein 1	Hematopoietic stem cell subset
CD319	CRACC; SLAM family member 7	Activated T
CD320	8D6; 8D6 antigen; FDC	N/A
CD321	JAM1; F11 receptor	Epithelium, endothelium
CD322	JAM2; junctional adhesion molecule 2	Epithelium, endothelium
CD324	E-cadherin; cadherin 1, type 1, E-cadherin (epithelial)	Epithelium
CDw325	E-cadherin; cadherin 2, type 1, N-cadherin (neuronal)	Neurons
CD326	Ep-CAM; tumor-associated calcium signal transducer 1	Epithelium
CDw327	siglec6; sialic acid binding Ig-like lectin 6	Cell-cell adhesion
CDw328	siglec7; sialic acid binding Ig-like lectin 7	Cell-cell adhesion
CDw329	siglec9; sialic acid binding Ig-like lectin 9	Cell-cell adhesion
CD331	FGFR1; fibroblast growth factor receptor 1	Fibroblasts
CD332	FGFR2; fibroblast growth factor receptor 2 (keratinocyte growth factor receptor)	Fibroblasts
CD333	FGFR3; fibroblast growth factor receptor 3 (achondroplasia, thanatophoric dwarfism)	Fibroblasts
CD334	FGFR4; fibroblast growth factor receptor 4	Fibroblasts
CD335	NKp46; NCR1, (Ly94); natural cytotoxicity triggering receptor 1	NK
CD336	NKp44; NCR2, (Ly95); natural cytotoxicity triggering receptor 2	NK
CD337	NKp30; NCR3	NK
CDw338	ABCG2; ATP-binding cassette, sub-family G (WHITE), member 2	Epithelium
CD339	Jagged-1; Jagged 1 (Alagille syndrome)	Broad

From: <http://mpr.nci.nih.gov/prow/>

LC: Langerhans cell; IDC: interdigitating dendritic cell; NK: natural killer; M: monocyte; Eo: eosinophil; Baso: basophil; G: granulocyte; DC: dendritic cell; FDC: follicular dendritic cell; PC: plasma cell; AML: acute myelogenous leukemia; RBC: red blood cell.

CD19 [12]. This molecule is not expressed on immature and mature T-lymphocytes, monocytic and granulocytic series, or erythroid precursors. However, CD19 is occasionally expressed in patients with AML [4–6].

CD20

CD20 is a membrane-embedded surface molecule which plays a role in the development and differentiation of

B-cells into plasma cells [5, 13]. It appears after HLA-DR, TdT, CD19, and CD10 expression and before cytoplasmic μ chain appearance in B-cell ontogeny. Similar to CD19, CD20 is a lineage-restricted molecule and is expressed on B-cells throughout B-cell differentiation prior to terminal differentiation of B-cells to plasma cell [13]. CD20 is expressed in the majority of B-cell lymphoid malignancies and some cases of plasma cell myeloma, T-cell leukemia/lymphomas, and AML [4–6].

CD21

CD21 is a receptor for EBV, C3d, C3dg, and iC3b [14]. Complement components may activate B-cells through CD21, which is part of a large signal-transduction complex that also involves CD19 and CD81 [5, 14]. This molecule is expressed on mature B-cells, from the stage when surface Ig is first expressed and then is lost upon activation. Follicular mantle zone B-cells and marginal zone B-cells express CD21. Also, follicular dendritic cells and subsets of thymocytes and T-cells express CD21 [4–6, 15].

CD22

CD22 is a single chain integral membrane molecule and a member of the immunoglobulin gene superfamily. It functions as an adhesion receptor and a signaling molecule, which appears to be involved in regulating the expression of surface IgM on peripheral B-cells and Ca^{++} flux in response to immunoglobulin signaling [16, 17]. Cytoplasmic CD22 is expressed at the earliest stages of B-cell differentiation, along with CD19 and prior to the expression of CD20. The majority of the TdT-positive precursor B-cells are also positive for cytoplasmic CD22. Expression of surface CD22 precedes or accompanies expression of surface IgM and/or IgD in mature B-lymphocytes, but it is lost in plasma cells. The neoplastic cells in various proportions of B-cell lymphoid malignancies, including precursor B-ALL, chronic lymphoid leukemias, and B-cell lymphomas, express CD22 [4–6, 18]. CD22 expression is particularly strong in hairy cell leukemia and prolymphocytic leukemia. T-cells and their malignant counterparts do not express CD22.

CD23

CD23 is an integral membrane glycoprotein involved in the regulation of IgE synthesis and pro-inflammatory activities, such as triggering the release of regulatory cytokines TNF, IL-1, IL-6, and GM-CSF by human monocytes [5, 19, 20]. It is expressed by activated B-cells, monocytes, follicular dendritic cells, and subsets of eosinophils and platelets [5]. Chronic lymphocytic leukemia (CLL) cells are positive for CD23, as are frequently follicular cell lymphoma cells [4, 5, 21]. The neoplastic cells in mantle cell and marginal zone B-cell lymphomas do not typically express CD23; neither do the neoplastic cells in plasma cell myeloma, ALL, T-cell, and myeloid malignancies [4–6].

CD24

CD24 is expressed on immature and mature B-cells except plasma cells. This molecule, however, is not lineage-restricted and is present on granulocytes and various benign and malignant epithelial cells [4–6, 22]. T-lymphocytes, monocytes, and erythroid precursors do not express CD24.

CD79

CD79 in association with surface Ig constitutes the B-cell antigen receptor complex on the surface of the B-lymphocytes

[5, 23]. CD79 consists of α and β heterodimers and plays a critical role in B-cell maturation and activation. The pattern of CD79 expression on B-cells is closely similar to that of CD19. CD79a is expressed initially in the cytoplasm prior to cytoplasmic μ heavy chain expression, and later on, after the expression of surface Ig, appears on the cytoplasmic membrane. CD79a is usually negative in CLL cells and plasma cells, whereas CD79b may be expressed in plasma cells and a significant proportion of CLL patients [5, 24]. CD79 is an excellent B-cell marker, but some cases of T-cell ALL and AML may react positively with anti-CD79 monoclonal antibodies [5, 25, 26].

CD138

CD138 is a transmembrane sulfate proteoglycan, which functions as a receptor for cell–matrix interactions [5, 27]. This molecule appears to be involved in the cellular organization in various tissues. Plasma cells adhere to type 1 collagen through CD138 and are the only hematopoietic cells that express this molecule. CD138 is expressed by various mesenchymal and epithelial cells, such as fibroblasts, endothelial cells, and stratified epithelia [5, 6, 28, 29].

OTHER B-CELL-ASSOCIATED MARKERS

CD5

The description of CD5 is briefed in the section “T-Cell-Associated CD Molecules.”

CD74

CD74 is an integral transmembrane molecule playing a role in intracellular sorting of MHC class II molecules [30, 31]. This molecule is expressed by most of the B-cells, particularly follicular center cells, mantle cells, and activated B-lymphocytes [5, 32]. A subpopulation of T-cells, macrophages, activated endothelial cells, and neoplastic plasma cells may also express CD74 [5, 32, 33].

CD103

CD103 is a membrane receptor which appears to play a role in the activation of intraepithelial lymphocytes [34]. It is expressed in >90% of intestinal intraepithelial lymphocytes and certain types of B- and T-cell lymphoid malignancies, such as *hairy cell leukemia* (B-cell), enteropathy-associated T-cell lymphoma, and adult T-cell leukemia/lymphoma [4, 6, 35, 36].

FMC7

FMC7 molecule binds to a particular conformation of the CD20 antigen, probably to a multimeric CD20 complex,

and it is detected only when CD20 antigen is present in high densities and in the postulated multimeric complex formation [37]. FMC7 is weakly expressed or is negative in CLL cells, and strongly positive in hairy cell leukemia and prolymphocytic leukemia [38, 39]. Antibodies against FMC7 are routinely used in flow cytometric studies for the diagnosis and classification of B-cell lymphoproliferative disorders.

T-CELL-ASSOCIATED CD MOLECULES

CD1

CD1 is a member of the immunoglobulin supergene family consisting of MHC class I-like glycoproteins [5]. So far, five distinct molecules of CD1 have been described: a, b, c, d, and e [5, 40]. The first three have been extensively used in diagnosis and classification of hematologic malignancies. CD1 molecules are expressed on thymocytes. They are absent on mature peripheral blood T-cells, but their cytoplasmic expression has been observed in activated T-lymphocytes. High levels of CD1a and less of CD1b and CD1c are present on Langerhans cells [41]. CD1c is expressed by the majority of cord blood and a subset of peripheral blood B-cells [5]. A subset of mantle zone and follicular center B-cells also express CD1c [4–6, 42, 43]. Follicular dendritic cells and monocytes/macrophages are CD1-negative.

CD2

CD2 is a transmembrane molecule and a member of the immunoglobulin supergene family and binds CD58, CD48, and CD59 [5, 44, 45]. The existence of this molecule was originally discovered by the ability of human T-cells to spontaneously bind sheep erythrocytes (E-rosette receptor) [5, 40]. CD2 plays an important role in T-cell activation, T- or NK-mediated cytotoxicity, apoptosis in activated peripheral T-cells, and the production of cytokines by T-cells [5, 44, 45]. It is expressed by thymic T-cells, peripheral T-cells, NK cells, and a subset of thymic B-cells [5, 44, 45]. CD2 is an excellent pan-T-cell marker and one of the earliest antigens which precedes CD1 but appears after CD7 on the T-cells [44–46]. However, some of the T-cell lymphoid malignancies, particularly peripheral T-cell lymphomas, may aberrantly lose CD2 expression. Also, some cases of AML, mainly promyelocytic type, may express CD2 [4–6].

CD3

CD3 is a complex structure composed of three different polypeptide dimmers: $\gamma\epsilon$, $\delta\epsilon$, and $\zeta\zeta$. CD3 in conjunction with T-cell receptor (TCR) makes the TCR complex (Figure 2.1). TCR molecules represent two different heterodimers: $\alpha\beta$ and $\gamma\delta$. The vast majority of T-cells bear TCR $\alpha\beta$ and only about 5% of T-cells express TCR $\gamma\delta$ [5, 6, 47]. The $\alpha\beta$ T-cells divide into CD4⁺ and CD8⁺ cells and are widespread and found in all hematopoietic and lymphoid tissues, whereas

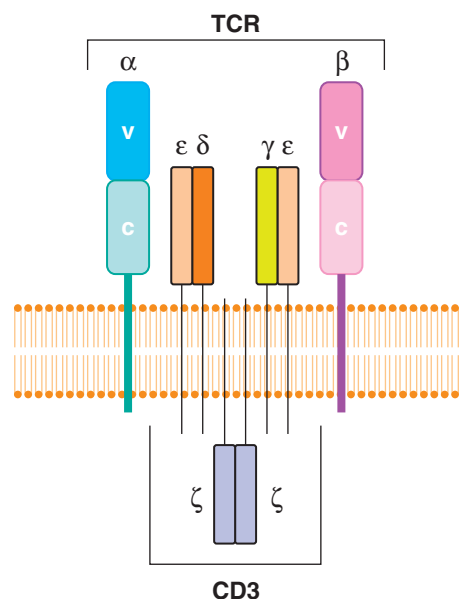


FIGURE 2.1 Schematic of TCR complex.

$\gamma\delta$ T-cells are negative for CD4 and CD8 and are primarily found in the spleen and intestinal epithelium [4–6]. NK cells do not express TCR complex but usually show cytoplasmic ϵ chain of CD3 [4–6].

Surface membrane CD3 is a pan-T-cell marker and is expressed by thymocytes and all mature T-cells of peripheral blood and lymphoid tissues. B-cells, granulocytic series, and monocytes/macrophages are all CD3-negative. NK cells are negative for membrane CD3 but may express cytoplasmic (ϵ chain) CD3 [5, 6]. Precursor T-lymphoblastic leukemias often show cytoplasmic CD3 expression.

CD4

CD4 is a membrane glycoprotein and a member of the immunoglobulin supergene family and a co-receptor in MHC class II-restricted T-cell activation [5, 48]. It also plays a role in the differentiation of thymocytes and the regulation of T-lymphocyte/B-lymphocyte adhesion [5, 48, 49]. CD4 is the primary receptor for HIV retroviruses [50]. CD4 is expressed in a large proportion of thymocytes (80–90%) and over 50% of the peripheral blood T-cells (helper/inducer subtype) [5, 48]. Most thymocytes coexpress CD4 and CD8. Monocytes, macrophages, and Langerhans cells (LC) express CD4. The majority of postthymic T-cell neoplasms are CD4-positive.

CD5

CD5 is a signal transducing molecule involved in tyrosine phosphorylation of intracellular proteins [5, 51]. It modulates signaling through the antigen-specific receptor complexes TCR and B-cell receptor (BCR) [5, 52]. CD5 is expressed at low density on thymocytes and at high density on all mature T-lymphocytes. It is also expressed at low density on a small subset of mature B-lymphocytes (B1a cells) which is expanded during fetal life and in several autoimmune

disorders. Certain B-cell lymphoid malignancies, such as chronic lymphocytic leukemia/small lymphocytic lymphoma and mantle cell lymphoma, express CD5 [4–6, 53].

CD7

CD7 is a transmembrane glycoprotein and a member of the immunoglobulin supergene family. It appears to play an essential role in T-cell and T-cell/B-cell interactions during early lymphoid development [5, 54]. CD7 is the earliest T-cell-associated molecule to appear in stem cells and prethymic stages and extends its expression all the way to the mature stages. The pluripotent stem cells, capable of differentiating to T-cells as well as erythroid, megakaryocytic, and myeloid precursors, may express CD7. This molecule is also present on most NK cells. A subpopulation of AML, particularly those with monocytic differentiation, may express CD7 [4–6, 55]. CD7 is an excellent marker for the detection of T-cell lymphoproliferative disorders. However, it is usually absent or weakly expressed in mycosis fungoides/Sezary syndrome and adult T-cell leukemia/lymphoma [4–6].

CD8

CD8 is a cell surface glycoprotein and a member of the immunoglobulin supergene family that is involved in the mediation of cell–cell interactions within the immune system [5, 56, 57]. This molecule is found on cytotoxic/suppressor T-lymphocytes and the majority of the thymocytes. Approximately 80–90% of the thymocytes and 35–45% of the peripheral blood lymphocytes express CD8. Most thymocytes coexpress CD8 with CD4. A subpopulation of NK cells also express CD8 [4–6].

CD45RA AND CD45RO

These molecules represent two different isoforms of the CD45 cluster. CD45 is typically expressed in all hematopoietic cells (a pan-leukocyte marker) [4–6]. CD45RA is expressed on naive/resting T-cells and medullary thymocytes, whereas CD45RO is detected on memory/activated T-cells, cortical thymocytes, monocytes/macrophages, and granulocytes [5, 58–60].

T-CELL RECEPTOR MOLECULES

As mentioned earlier, TCR heterodimers, $\alpha\beta$ and $\gamma\delta$, in association with CD3 make the TCR complex (Figure 2.1). The vast majority of T-cells bear TCR $\alpha\beta$ and only about 5% of T-cells express TCR $\gamma\delta$ [5, 6, 61–63]. The $\alpha\beta$ T-cells are widespread and are found in all hematopoietic and lymphoid tissues, whereas $\gamma\delta$ T-cells are primarily found in the spleen and intestinal epithelium. NK cells do not express TCR; neither do the B-cells, monocytes/macrophages, or granulocytic cells.

OTHER T-CELL-ASSOCIATED MARKERS

CD26 is a T-cell co-stimulatory molecule with dipeptidyl peptidase activity and is considered as a T-cell activation molecule [64, 65]. CD26 expression is lost in the Sezary cells [66].

CD246 or anaplastic lymphoma kinase (ALK) is expressed by the neoplastic cells in anaplastic large cell lymphomas [67, 68].

CD247 is a component of the TCR complex and is expressed by T-cells [69].

CD CLUSTERS ASSOCIATED WITH LARGE GRANULAR LYMPHOCYTES

CD16

CD16 is a low affinity IgG receptor expressed on large granular lymphocytes (LGL) of both NK- and T-cell types [5, 70]. This molecule is involved in antibody-dependent cell-mediated cytotoxicity [70, 71]. Approximately 15–20% of the peripheral blood lymphocytes and a much smaller fraction (<5%) of bone marrow lymphocytes express CD16. CD16 is also expressed on granulocytes, tissue macrophages, and a subset of monocytes, eosinophils, and dendritic cells [5, 72, 73]. CD16 expression is reduced or lost in paroxysmal nocturnal hemoglobinuria (PNH) due to the structural abnormality of glycosyl-phosphatidyl-inositol (GPI) [74, 75].

CD56

CD56 is a transmembrane-anchored glycoprotein and a member of the immunoglobulin supergene family [5, 76–78]. It functions as an adhesion molecule on neural and NK cells, and a subset of T-cells. NK cells are divided into CD56^{bright} and CD56^{dim} [5, 79]. The CD56^{dim} subset represents about 90% of the NK cells, is CD16-positive, and contains higher levels of granzyme A and perforin, two molecules involved in exocytosis-mediated cytotoxicity [5, 78]. The CD56^{bright} NK cells are CD16^{dim} or negative. A subset of dendritic cells, known as plasmacytoid dendritic cells, coexpress CD56 and CD4 [80].

CD56 is an excellent marker for the detection of NK cells and T-LGL lymphoproliferative disorders, but it is also expressed in some cases of plasma cell myelomas, AML, and ALL [4–6]. Hematodermic neoplasms (blastic NK cell lymphomas) coexpress CD4 and CD56 and appear to be the plasmacytoid dendritic cell origin [80]. Neuroectodermal tumors, such as small cell carcinoma of lung, neuroblastoma, medulloblastoma, and astrocytoma, are CD56-positive [5, 81, 82].

CD57

The CD57 molecule is a glycoprotein expressed on NK cells, T-cell subsets, and some cells of neuroectodermal origin [5, 83]. The proportion and absolute number of CD57-positive

cells in peripheral blood increases with age. In adults, CD57 is expressed by 10–25% of the peripheral blood mononuclear cells [5, 83–85]. The CD57-positive cells are proliferation incompetent and most of them coexpress CD8. A small fraction of CD4⁺ T-cells express CD57 and appear to be associated with chronic inflammatory conditions, such as tuberculosis, malaria, and AIDS [5]. The CD57⁺CD4⁺ T-cells constitute a major subset of T-cells in the germinal center of the lymphoid tissues. Approximately 40% of the CD16-positive cells coexpress CD57 [5, 84]. The CD16⁺CD57⁺ subset demonstrates strong cytotoxic activities.

CD57 is a good marker for the detection of LGL disorders. The CD4⁺CD57⁺ cells are increased in lymphocyte predominance Hodgkin lymphoma and chronic inflammatory conditions [5, 6]. CD57 is positive in a wide variety of tumors of neuroectodermal or mesenchymal origin [5, 86].

OTHER NK/LGL-ASSOCIATED MARKERS

The 8th International Workshop on HLDA in December 2004 designated 95 new CD clusters which include several NK-associated markers:

CD158 or killer cell inhibitory receptor is expressed by NK cells [87, 88].

CD161 is expressed on most NK cells and a subset of CD4⁺ and CD8⁺ T-cells [89, 90].

CD335 was previously known as NKp46, NCR1, (Ly94), or natural cytotoxicity triggering receptor 1 [91].

CD336 was previously referred to as NKp44, NCR2, (Ly95), or natural cytotoxicity triggering receptor 2 [92].

CD337 was previously known as NKp30, NCR3, or natural cytotoxicity triggering receptor 3 [93].

GRANULOCYTIC/MONOCYTIC-ASSOCIATED CD MOLECULES

CD13

CD13 is an integral membrane zinc-binding aminopeptidase which is expressed on the surface of about 40% of granulocytes/monocytes precursors and mature granulocytic/monocytic cells [5, 94]. This molecule is also expressed on endothelial cells, bone marrow stromal cells, osteoclasts, and a small proportion of LGL. This molecule is not expressed in other lymphocytes, erythroid cells, or platelets. CD13 is commonly used as a pan-myeloid marker for the diagnosis of AML [4–6, 95]. However, about 5–15% of acute lymphoid leukemias also express CD13 [4–6, 96]. The epithelia of renal proximal tubules and bile duct canaliculi may express CD13 [5, 94].

CD14

CD14 is a lipopolysaccharide-binding protein, which functions as an endotoxin receptor [5, 97]. It is anchored to the

cell surface by linkage to GPI. CD14 is strongly expressed on the surface of monocytes and most tissue macrophages, and weakly expressed on the surface of granulocytes [4–6]. Myeloid precursors and monoblasts are negative for CD14. A small proportion of peripheral blood lymphocytes and mantle cells may weakly express CD14 [5]. T-cells, dendritic cells, and platelets are CD14-negative, though CD14 expression has been reported in non-myeloid cells [5, 98]. CD14 expression is reduced or lost in PNH due to the structural abnormality of GPI [99]. Anti-CD14 monoclonal antibodies are frequently used for the identification of leukemias with monocytic differentiation [4–6].

CD15

CD15 is a carbohydrate-based molecule expressed in the granulocytic series past the myeloblast stage [5]. A significant proportion of monocytes, a minority of macrophages/histiocytes, and a wide variety of epithelial cells and their malignant counterparts also express CD15 [4–6, 100, 101]. Erythroid precursors, B-cells, T-cells, and NK cells are CD15-negative. Reed–Sternberg cells and activated T-cells may express CD15 [4–6, 102].

CD33

CD33 is a sialoadhesin molecule and a member of the immunoglobulin supergene family [5, 103]. It is expressed by myeloid stem cells (CFU-GEMM, CFU-GM, CFU-G, and E-BFU), myeloblasts and monoblasts, monocytes/macrophages, granulocyte precursors (with decreasing expression with maturation), and mast cells [5, 103]. Mature granulocytes may show a very low level of CD33 expression. This molecule is not expressed in erythrocytes, platelets, B-cells, T-cells, and NK cells. CD33 is an excellent myeloid marker and is commonly used for the diagnosis of AML. However, approximately 10–20% of precursor B-ALL may express CD33 [4–6, 103, 104].

CD64

The CD64 molecule is a member of the immunoglobulin supergene family and functions as an FcIgG receptor [5, 105]. It is expressed by monocytes/macrophages, myeloid precursors, and follicular dendritic cells. CD64 and CD14 are considered good monocyte/macrophage-associated markers and are commonly used in flow cytometric studies to identify leukemias with myelomonocytic differentiation [4–8]. CD64 appears to be more sensitive but less specific monocytic marker than CD14 [106, 107]. Langerhans cells, interdigitating dendritic cells, B-cells, T-cells, NK cells, and erythroid and megakaryocytic lineages are CD64-negative.

CD68

CD68 is a sialomucin and a member of the scavenger receptor supergene family [5, 108]. This molecule is expressed by

monocytes and macrophages as well as subsets of CD34-positive hematopoietic stem cells, dendritic cells, neutrophils, basophils, and mast cells [5, 109, 110]. Activated T-cells and a proportion of mature B-cells may also express CD68, which usually appears as a dot-like cytoplasmic or finely granular positivity by immunohistochemical techniques. Some non-hematopoietic cells, such as epithelium of renal tubules, may show CD68 positivity [5, 111].

OTHER GRANULOCYTIC/MONOCYTIC-ASSOCIATED MARKERS

CD88 is a C5a receptor and is expressed by granulocytes, monocytes, mast cells, and subsets of dendritic cells [112, 113].

CD114 is the receptor for granulocyte colony-stimulating factor (G-CSF) [114–116]. It is expressed by cells of the granulocytic lineage in all stages of differentiation and is found in various proportions of monocytes, endothelial cells, and trophoblastic cells [116–118].

CD115 is the receptor for macrophage colony-stimulating factor (M-CSF) and is primarily expressed on cells of the monocyte/macrophage lineage [119].

CD116 is the α chain subunit of the GM-CSF receptor and is expressed by various myeloid cells including macrophages, neutrophils, eosinophils, and dendritic cells [120].

ERYTHROID-ASSOCIATED CD MOLECULES

CD235

CD235 molecules represent glycoproteins A and B, the two major sialoglycoproteins of the human erythrocyte membrane [121, 122]. These molecules bear the antigenic determinants for the MN and Ss blood groups [122, 123]. Monoclonal antibodies against glycoprotein A (GPA) are frequently used in immunophenotypic studies for the identification of erythroid precursors in hematologic disorders [4, 124].

CD238

The CD238 molecule is the Kell blood group transmembrane protein [125].

CD240

CD240 represents the CE and D antigens of the Rh blood group system, the second most clinically significant, and the most polymorphic of the human blood groups [126].

CD242

CD242 is an intercellular adhesion molecule (ICAM4) and represents the Landsteiner–Wiener (LW) blood group antigen(s) [127].

OTHER ERYTHROID-ASSOCIATED MARKERS

CD71 is the transferrin receptor and is expressed on all proliferating cells [128]. It is also expressed by erythroid precursors which need iron for the synthesis of heme molecules [128, 129]. CD71 in conjunction with glycoprotein A (GLPA) is a helpful marker in the identification of erythroid precursor cells in hematologic disorders [4, 129].

Anti-hemoglobin antibodies are routinely used for immunophenotypic studies of erythroid precursors.

MEGAKARYOCYTE/PLATELET-ASSOCIATED CD MOLECULES

CD42

CD42 complex (a, b, c, and d) is restricted to the megakaryocytic lineage and platelets [130]. This complex facilitates adhesion of the platelets to the subendothelial matrices. Absence of the CD42 complex leads to the Bernard–Soulier syndrome [131]. Anti-CD42 monoclonal antibodies are routinely used for identification of megakaryoblasts and immature megakaryocytes in myeloproliferative disorders and myeloid leukemias [4, 132].

CD41 and CD61

CD41 (platelet glycoprotein IIb) and CD61 (platelet glycoproteins IIIa) form a calcium-dependent heterodimeric complex [133]. This glycoprotein complex (GPIIb–IIIa) binds plasma proteins, such as fibrinogen, fibronectin, von Willebrand factor, and vitronectin, and plays a critical role in platelet aggregation [134]. Hereditary defects of the GPIIb–IIIa receptor cause Glanzmann's thrombasthenia [131, 134]. Similar to CD42, anti-CD41 and CD61 monoclonal antibodies are frequently used for identification of megakaryocytic precursors in myeloproliferative disorders and myeloid leukemias [4].

OTHER MEGAKARYOCYTE/PLATELET-ASSOCIATED MARKERS

CD110 or thrombopoietin receptor (TPO-R) is expressed on the megakaryocytic precursors and platelets, hematopoietic stem cells, and some of the hematopoietic precursors [135].

Factor VIII is another useful megakaryocytic marker used for identification of megakaryocytic precursors in myeloproliferative disorders and myeloid leukemias.

PRECURSOR-ASSOCIATED CD MOLECULES

CD34

CD34 is a transmembrane glycoprotein expressed on early lymphohematopoietic stem cells, progenitor cells, and endothelial cells [5, 136, 137]. Also, embryonic fibroblasts and some cells in fetal and adult nervous tissue are CD34-positive. Almost all pluripotent and committed stem cells in colony-forming assays express CD34 [5, 136]. The uncommitted progenitor cells are CD38-negative, and the committed ones are CD38-positive. In normal conditions, CD34⁺ cells account for about 1–2% of the total bone marrow cells. The TdT⁺ precursor B-cells (hematogones) are also positive for CD34. Approximately 40% of AML and over 50% of ALL express CD34 [4–6, 138, 139].

CD38

CD38 is a multifunctional ectoenzyme widely expressed in hematopoietic cells [5, 140, 141]. It plays a role in the regulation of cell activation and proliferation. It is expressed in committed hematopoietic stem cells and other hemopoietic precursors during early differentiation and activation [4, 141]. Very early erythroid and myeloid cells, precursor B-cells, thymocytes, and activated T-cells and NK cells express CD38 [4, 142]. It is also expressed at high levels on plasma cells [4, 143].

CD90

The CD90 molecule is a member of the immunoglobulin supergene family and is expressed by 10–40% of CD34⁺ cells in bone marrow [144, 145]. The CD34⁺/CD90⁺ cells probably represent the most primitive hematopoietic progenitor cells [144, 145]. CD90 is also expressed in fibroblasts and other stromal cells.

CD99

CD99 is a transmembrane protein involved in homotypic cell adhesion, apoptosis, vesicular protein transport, and differentiation of T-cells [146, 147]. Its expression has been reported in acute lymphoid leukemias, germ cell tumors, and tumors of neuroectodermal origin [146–148].

CD117

CD117 (c-kit) is a tyrosine kinase receptor and a member of the immunoglobulin supergene family [149]. It is expressed in most of the hemopoietic stem and CD34⁺

progenitor cells, and mast cells. The majority of AML cells are also CD117-positive. Plasma cells may also express CD117 [150]. CD117 is an excellent marker for the detection of mast cell disorders and identification of myeloblasts in acute leukemias [151–153].

OTHER PRECURSOR-ASSOCIATED MARKERS

TdT (terminal deoxynucleotidyl transferase) is a DNA polymerase present in precursor T- and B-cells and thymocytes. Anti-TdT antibodies are routinely used for the detection of precursor B- and T-acute lymphoid leukemias/lymphomas and lymphoid blast transformation in chronic myeloid leukemia (CML) [4–6]. A small proportion of AMLs are also TdT-positive [154].

OTHER MARKERS ROUTINELY USED IN HEMATOPATHOLOGY

CD11

CD11a, b, and c are components of heterodimer CD11/CD18 adhesion molecules [5, 155]. CD11a is a pan-leukocyte marker and is expressed by B- and T-lymphocytes, monocytes, macrophages, neutrophils, basophils, and eosinophils [5]. CD11b is strongly expressed by most of the granulocytes, monocytes/macrophages, and NK cells, and subsets of B- and T-cells [5]. CD11c expression is high in monocytes/macrophages, NK cells, and hairy cells, moderate in granulocytes, and weak in lymphocyte subsets [4–6].

CD30

The CD30 molecule is a member of the TNF receptor family and appears to be involved in TCR-mediated cell death [5, 156]. It is expressed by Reed–Sternberg cells and Hodgkin cells, cells of anaplastic large cell lymphoma (ALCL), and activated T-, NK-, and B-cells, and monocytes [4–6, 157, 158]. Some cases of embryonal carcinoma and mixed germ cell tumors also express CD30 [159, 160].

CD43

CD43 is a sialomucin transmembrane molecule expressed at high levels, on all leukocytes except most resting B lymphocytes [5, 161]. In hematopathology, CD43 is often considered as a T-cell-associated marker, because it is expressed by over 95% of thymocytes and peripheral blood T-cells. Interdigitating dendritic cells, Langerhans cells, epithelioid histiocytes, and multinucleated giant cells express CD43, whereas follicular dendritic cells and sinus histiocytes of the lymph nodes are usually CD43-negative [4–6].

CD43 may be expressed in mantle cell lymphoma, mastocytosis, and some cases of plasma cell disorders [4–6]. Loss or defect of CD43 has been reported in lymphocytes of patients with Wiskott–Aldrich syndrome [162, 163].

CD55

CD55 or decay accelerating factor (DAF) binds C3b and C4b to inhibit formation of the C3 convertases [164]. It is anchored to the GPI in the cell membrane, and, therefore, its expression is reduced or lost in patients with PNH [165, 166]. It is widely expressed on cells throughout the body, including hematopoietic cells.

CD59

CD59 is also a GPI-anchored molecule and inhibits formation of membrane attack complex (MAC), thus protecting cells from complement-mediated lysis [167]. Similar to CD55, CD59 expression is reduced or lost in patients with PNH [165, 166]. It is widely expressed on cells throughout the body, including hematopoietic cells.

Ki-67

Ki-67 is a proliferation-associated molecule [168]. Its expression is upregulated during the S phase of the cell cycle and is maximized during mitosis [169]. Anti-Ki-67 antibodies are used for the estimation of proliferating index in lymphoid malignancies.

PAX5

The *PAX5* gene encodes the B-cell lineage-specific activator protein (BSAP) which is a member of the highly conserved paired box (PAX)-domain family of transcription factors [170]. It plays a crucial role in B-cell development and commitment of the bone marrow multipotent progenitor cells to the B-lymphoid lineage [171]. Antibodies to PAX5 are used for the diagnosis of lymphoid malignancies, particularly precursor B-ALL [172, 173]. Neuroendocrine neoplasms and t(8;21)-AML may also express PAX5 [173].

IMMUNOGLOBULIN TRANSCRIPTION FACTORS

Oct1, Oct2, and BOB.1/OBF.1

Oct1 and Oct2 and their co-activator BOB.1/OBF.1 regulate immunoglobulin gene transcription [174]. Antibodies raised against these molecules are used for the characterization of certain types of B-cell lymphoid malignancies [174, 175]. They are also used for the identification of the L&H cells in the lymphocyte predominant Hodgkin lymphoma. Oct2 and BOB.1/OBF.1 are expressed in L&H cells but not in Reed–Sternberg cells of classical Hodgkin lymphomas [6].

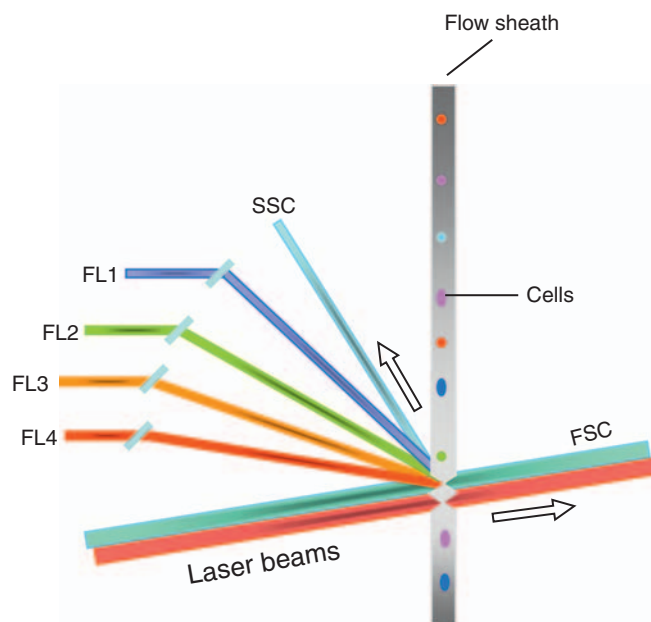


FIGURE 2.2 A simplified diagram of a flow cytometer. FSC: forward scatter, SSC: side scatter, FL1, -2, -3, and -4 represent various types of fluorochromes.

ZAP-70

ZAP-70 is a tyrosine kinase that plays a role in TCR-linked signal transduction [176,177]. This molecule is expressed in T-cells and NK cells, precursor B-acute lymphoblastic leukemia cells, and CLL cells, particularly in those cases with unmutated IgV_H genes [177, 178]. ZAP-70 expression in CLL appears to be associated with a worse prognosis in terms of progression and survival [178].

PRINCIPLES OF FLOW CYTOMETRY

Flow cytometry is now considered an integral component of immunophenotyping in hematopathology. Access to a huge number of antibodies against CD molecules and high quality and diverse fluorochromes, sophisticated and user-friendly flow cytometry instruments, advanced software, improved gating strategies, and multiparameter interpretation approaches have made flow cytometry a powerful method of immunophenotyping [179–182].

The flow cytometer is basically a particle analyzer. It measures cell properties when a stream of single cell (or particle) suspension passes through a laser beam (Figure 2.2). The cell size, texture (granularity), and membrane-associated, cytoplasmic or nuclear molecules that are marked by different fluorochromes are measured by a set of optical detectors and analyzed [179–182]. A fluorochrome is a chemical which can absorb energy from an excitation source (laser light) and emits photons at a longer wavelength (fluorescence). Most flow cytometry instruments are able to measure and analyze at least six parameters simultaneously: cell size

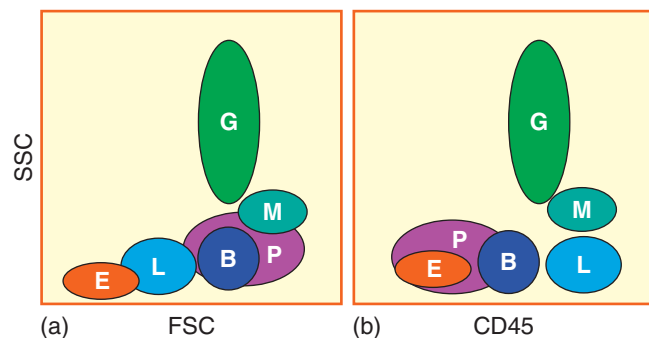


FIGURE 2.3 Patterns of cell aggregation in flow cytometry analysis of FSC/SSC (a) and CD45/SSC (b). E: erythroid precursors, L: lymphocytes, B: blasts, M: monocytes, G: granulocytes, and P: plasma cells.

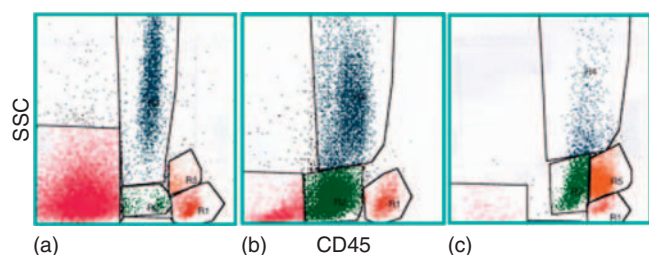


FIGURE 2.4 Multicolor cell plot presentations of flow cytometry demonstrating example patterns of a plasma cell myeloma (a, red cluster), an acute lymphoblastic leukemia (b, green cluster), and an acute myelomonocytic leukemia (c, green and red clusters).

depicted by forward scatter (FSC) laser light, cell granularity represented by side scatter (SSC) laser light, and at least four different fluorochromes defining four different molecular characteristics of the cells passing through the instrument (Figure 2.1). The basic principles of flow cytometry are briefly discussed later.

GATING

Gating refers to the selection of a population of cells in an electronic window. Cells sharing similar electronic signal tend to be aggregated together in the electronic windows. For example, in flow cytometric study of peripheral blood leukocytes, lymphocytes which are small and non-granular appear as an aggregate in the lower left section of the FSC versus SSC electronic window (Figure 2.3). Currently, the recommended gating strategy for hematopoietic tissues is a combination of FSC versus SSC and CD45 versus SSC (Figure 2.3). CD45 is strongly expressed by lymphocytes and monocytes while erythroid precursors are CD45-negative. Blast cells are CD45^{dim} and plasma cells are CD45-negative to CD45^{dim}. This gating strategy is extremely helpful in separating blast cells from non-blast cells, lymphoid cells from non-lymphoid cells, and monocytes from

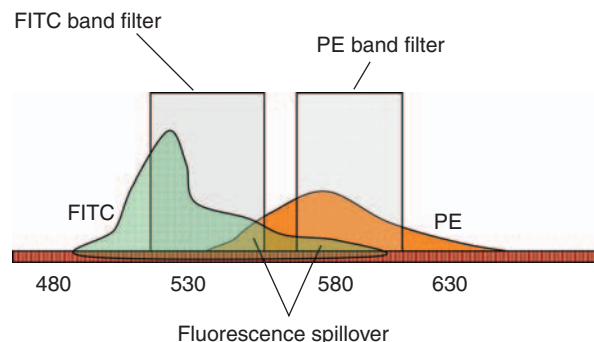


FIGURE 2.5 An example of fluorescence spillover between FITC and PE. Adapted from Wulff S. *Guide to Flow Cytometry*, DakoCytomation.

granulocytes (Figures 2.3 and 2.4). It also helps to distinguish the normal patterns of expression from the abnormal ones. Proper gating is a critical step in data analysis and interpretation of the results in flow cytometry.

In order to gate the right cell population for the flow cytometric analysis, we strongly recommend microscopic review of the samples and access to clinical information prior to the selection of monoclonal antibodies and gating processes.

COMPENSATION

When multiple fluorochromes are used, there is a possibility of fluorescence interference due to the overlapping emission spectra. For example, both fluorescein isothiocyanide (FITC) and phycoerythrin (PE) are excited at 488 nm, but their maximal emission peaks are at 520 and 576 nm, respectively [181, 183]. However, since the emission wavelength of these fluorochromes are relatively broad, there is an overlap between the emitted FITC and PE fluorochromes, even when the proper filters are used to limit this overlap (Figure 2.5). The currently available flow cytometry programs are able to correct (eliminate) the fluorescence overlap. This corrective measure is called compensation. Compensation is one of the most challenging technical aspects in multicolor flow cytometry. The use of new tandem fluorochromes, the emergence of newly developed compensation software, and publications providing guidelines for the standardization of compensation have significantly improved this process [183–186]. In practice, for monitoring compensation, we usually utilize two mutually exclusive markers such as CD4 and CD8 in peripheral blood samples. All peripheral blood T-cells express CD3, but they are positive for either CD4 or CD8 (Figure 2.6).

DATA ANALYSIS

Sophisticated flow cytometry instruments in conjunction with powerful and user-friendly software programs offer great

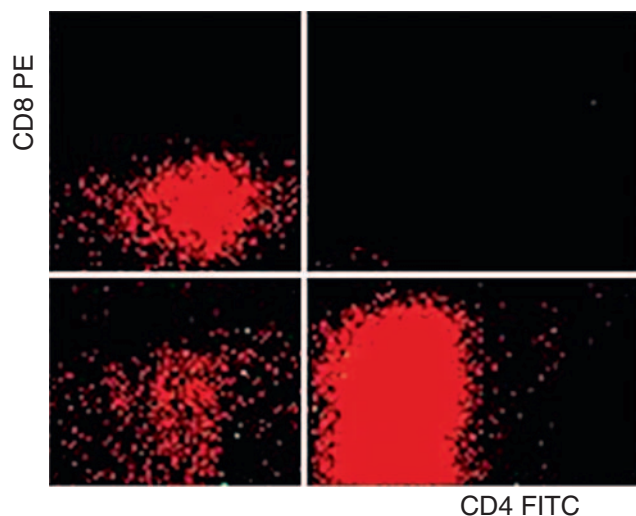


FIGURE 2.6 In practice, for monitoring compensation, two mutually exclusive markers such as CD4 and CD8 are utilized in a peripheral blood sample.

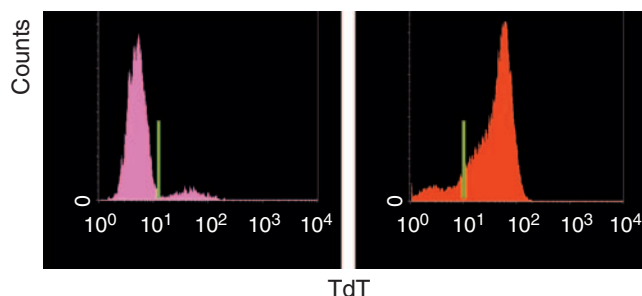


FIGURE 2.7 Histograms for a single parameter usually depict fluorescent intensity versus cell count. The TdT-negative and TdT-positive samples are demonstrated in left and right, respectively.

opportunities for hematopathologists, immunologists, and researchers to rapidly acquire data and analyze the results on large cell populations. Most available instruments are able to process >50,000 cells per second and detect at least six parameters (two light scatter and four fluorescent signals) simultaneously. Software programs provide a wide variety of options for the evaluation and analysis of the signals, including data collection on logarithmic or linear scales, and different options for histograms. The logarithmic scale is the preferred scale for most flow cytometric immunophenotypic studies. Histograms for a single parameter usually depict fluorescent intensity versus cell counts (Figure 2.7). Dot and density plots provide simultaneous information for two parameters. Two-parameter dot plot histograms depict four quadrants. The lower left quadrant represents negative cell cluster, the upper left quadrant shows the cell population positive for one parameter, the lower right quadrant depicts cells positive for the second parameter, and the upper right quadrant represents cells that coexpress both parameters

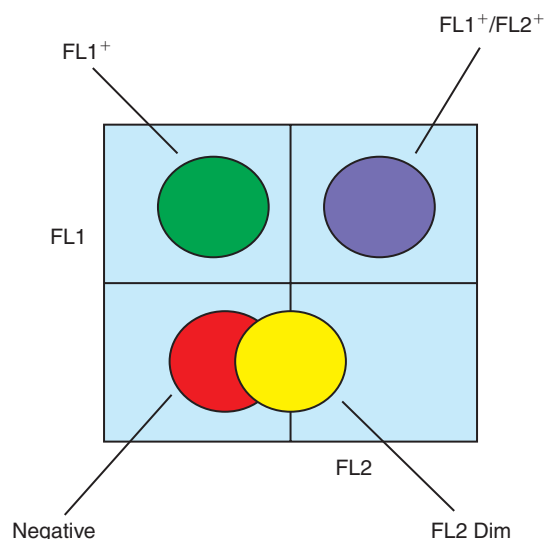


FIGURE 2.8 Two-parameter dot plot histograms depict four quadrants: Lower left: negative; upper left: positive for one parameter; lower right: positive for the second parameter; and upper right: positive for both parameters.

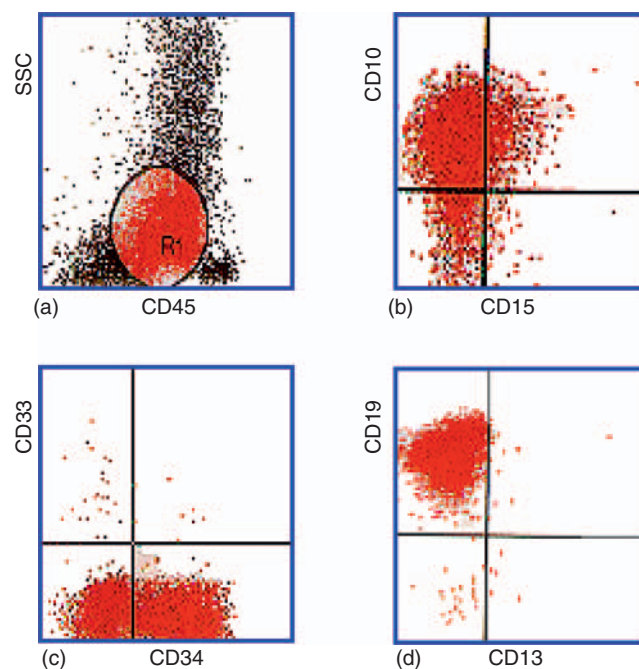


FIGURE 2.9 An example of two-parameter dot plot analysis showing a population of CD45^{dim} blast cells (a) expressing CD10 (b), CD34 (c), and CD19 (d).

(Figures 2.8 and 2.9). Contour histograms display the data as a series of encircling lines correlating with cellular density and distribution (Figure 2.10). Most programs also allow us to compare data from multiple samples by simultaneously overlaying their single parameter histograms on top of one another.

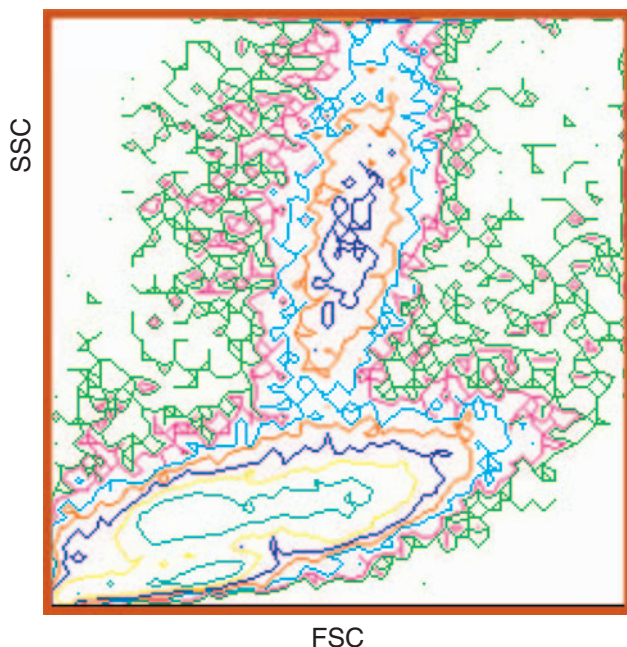


FIGURE 2.10 Counter histograms display the data as a series of encircling lines correlating with cellular density (higher in the center) and cell clusters.

QUALITY CONTROL AND QUALITY ASSURANCE ISSUES

Similar to all other instruments in the clinical laboratories, flow cytometry has its own quality control (QC) and quality assurance (QA) issues [183–187]. Many steps are involved in various aspects of flow cytometry, such as the optimization of instrument function, sample processing, acquiring and analyzing data, and reporting the results. Flow cytometers should be calibrated with samples consisting of a mixture of blank and predefined fluorescence-labeled microbeads. The performance of various components, such as fluidics, optical filters, multiplier tubes, and lasers, should be checked on a regular basis. Standardized protocols for each step of the process should be implemented to ensure reliable results, including verification of accuracy of the results with known samples.

PRINCIPLES OF IMMUNOHISTOCHEMISTRY

Immunohistochemistry has become a routine staining technique in most pathology laboratories. Enzyme-conjugated antibodies are used for the demonstration of antigens in tissue sections, smears, and cytospin preparations [188–190]. Horse radish peroxidase and/or alkaline phosphatase are the most frequently used enzymes for signal generation. Sections from frozen or fixed tissues are used [190–193].

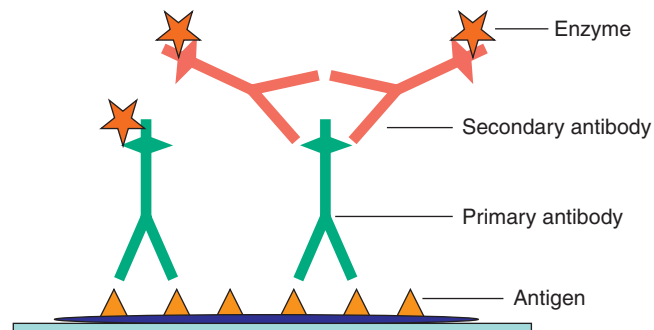


FIGURE 2.11 Schematic of immunohistochemical techniques demonstrating direct and indirect immunoenzyme methods. Adapted from Ref. [195].

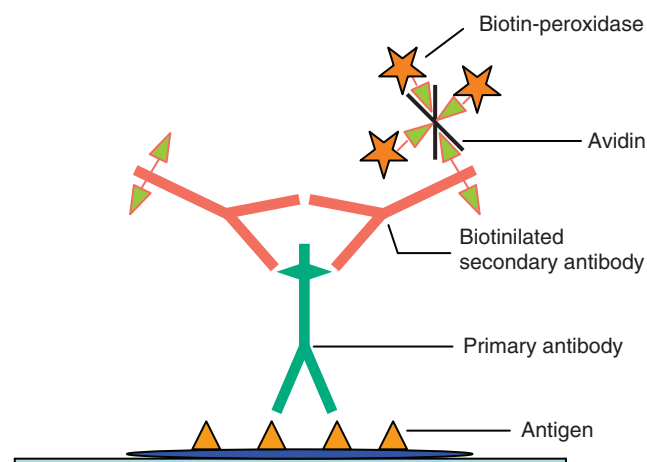


FIGURE 2.12 Schematic of immunohistochemical techniques demonstrating an indirect method using biotin-avidin-enzyme complexes. Adapted from Ref. [195].

Archival tissue blocks are sectioned and deparaffinized and then properly heated for epitope retrieval [188, 189, 194]. After blocking the endogenous peroxidase, the primary antibody (1–5 μ g) is applied with proper incubation time (\sim 30 min) and then the enzyme-conjugated or biotinylated secondary antibody is added (Figure 2.11) [195]. To amplify the signals, biotin-avidin-enzyme or biotin-streptavidin-enzyme complexes are used (Figure 2.12) [195]. Currently, there are automated machines available for performing single or dual immunohistochemical stains (Figure 2.13).

Immunohistochemistry provides information regarding pattern, intensity, and location of antigen(s) in tissues and cells. It is used in hematopathology for diagnosis and classification of leukemias and lymphomas, and differential diagnosis of primary hematopoietic neoplasms from non-neoplastic hematopoietic disorders and metastatic tumors [188, 189] (Figures 2.14 and 2.15). For example, most of the acute lymphoid leukemias are CD10, TdT, and HLA-DR positive, whereas these markers are not expressed in neuroblastoma, rhabdomyosarcoma, or Ewing's sarcoma. Immunohistochemical technique

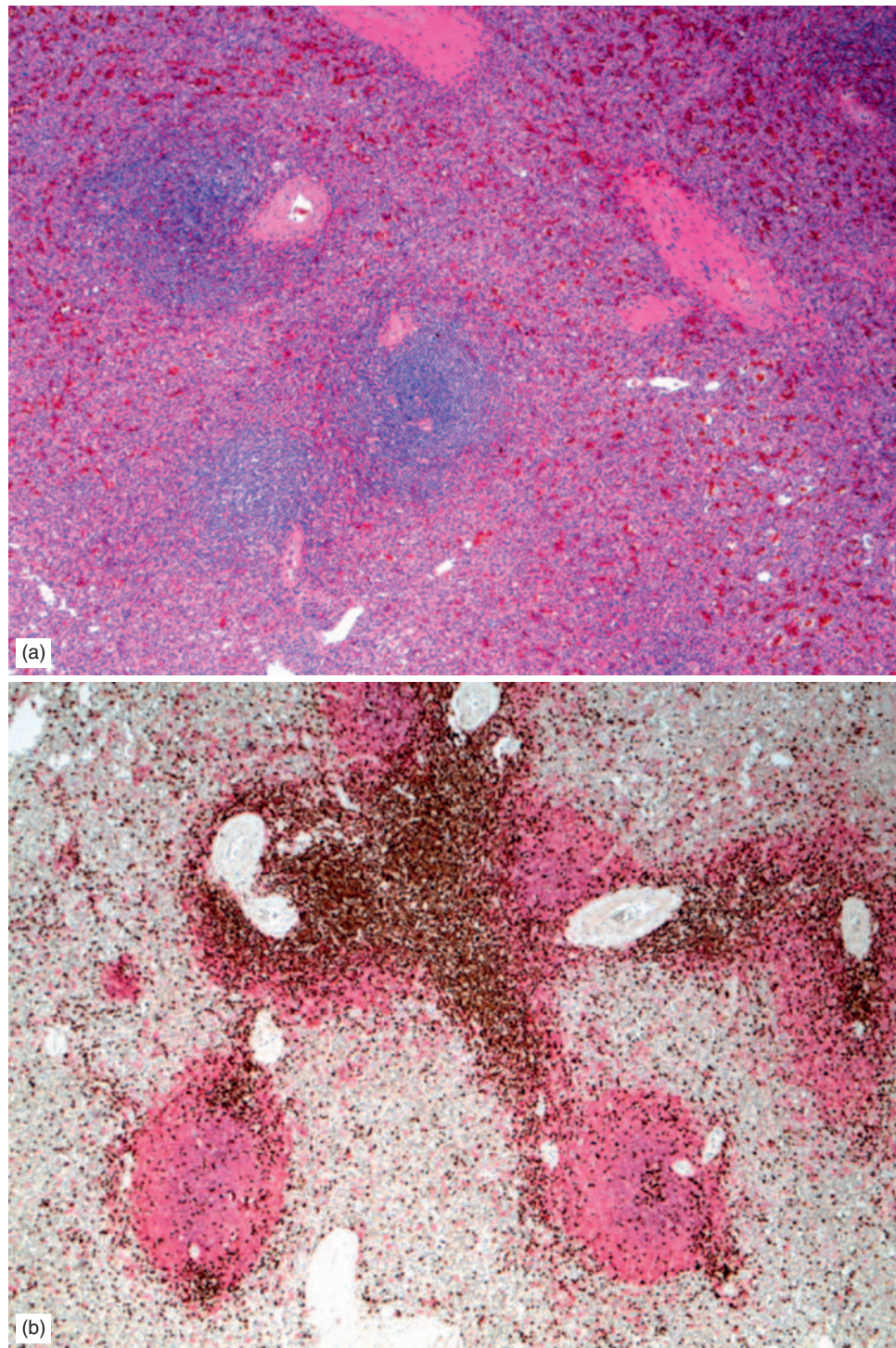


FIGURE 2.13 (a) An H&E section of spleen demonstrating white and red pulps. (b) Dual immunohistochemical stains showing T (CD3-positive, brown) and B (CD20-positive, pink) cells.

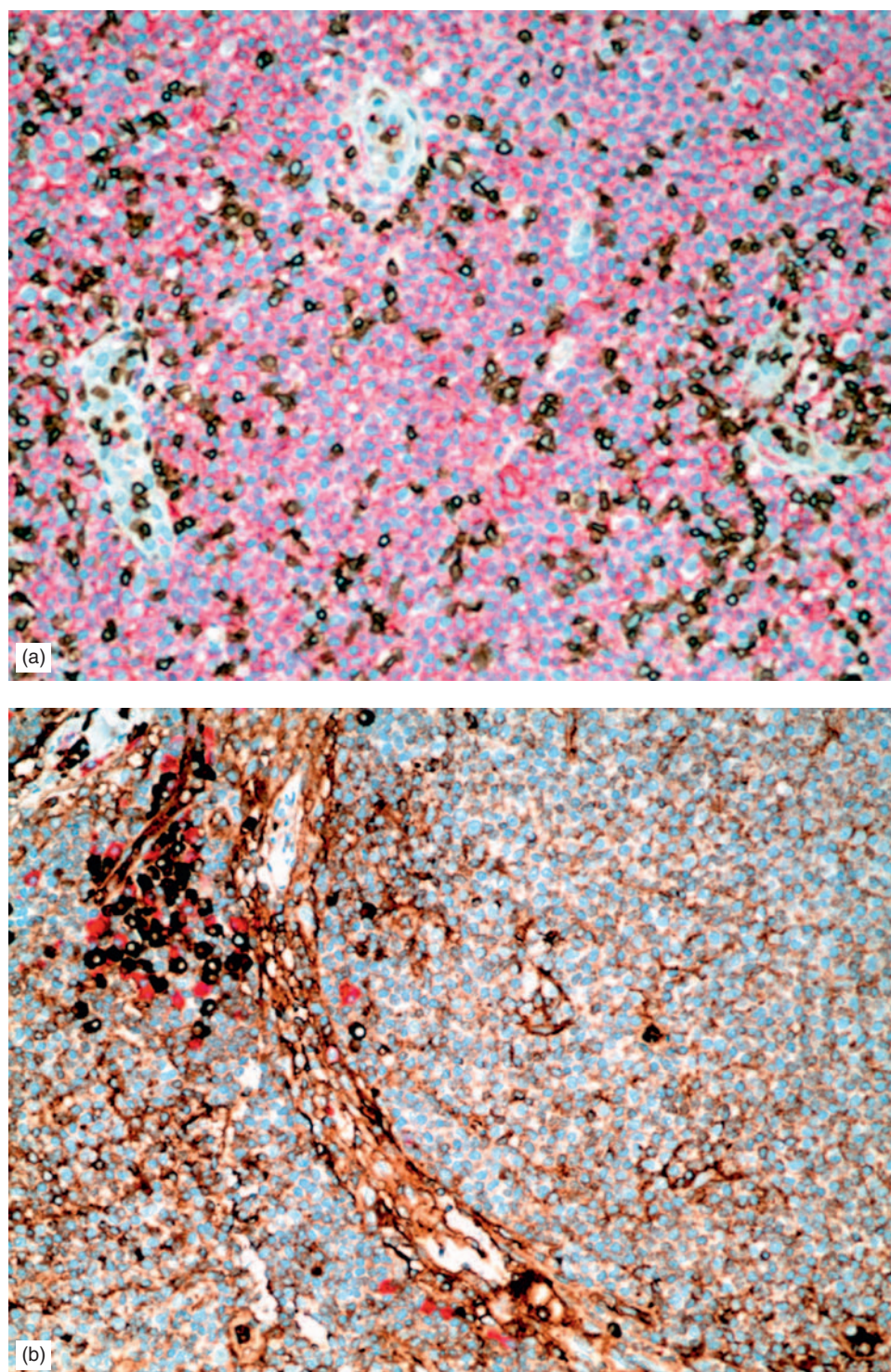


FIGURE 2.14 (a) Dual immunohistochemical staining of a lymph node section from a patient with B-cell lymphoma demonstrating large numbers of B-cells (CD20⁺, pink) and scattered T-cells (CD3⁺, brown). (b) Dual kappa (brown) and lambda (pink) staining shows a cluster of polyclonal plasma cells and sheets of kappa-positive lymphocytes.

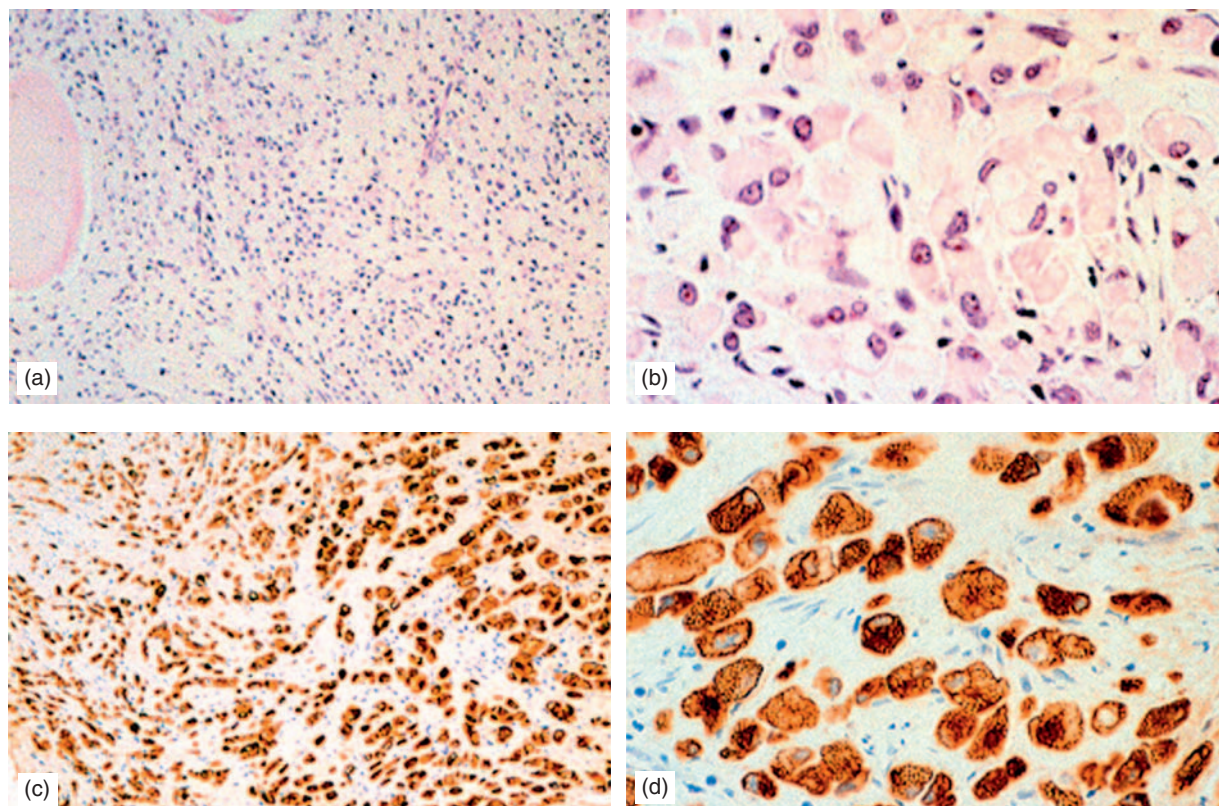


FIGURE 2.15 Metastatic adenocarcinoma simulating a histiocytic infiltrate (a and b: H&E; low and high power views). Immunohistochemical stain for cytokeratin shows numerous positive cells (c and d, low and high power views). From Naeim F. (2001). *Atlas of Bone Marrow and Blood Pathology*. W.B. Saunders, Philadelphia, by permission.

may help to detect occult metastatic lesions, such as metastatic breast carcinomas and neuroblastomas, or to identify their tissue of origin [196–198]. For example, metastatic prostatic carcinomas are positive for prostatic acid phosphatase and prostate-specific antigen (PSA), and metastatic rhabdomyosarcomas may demonstrate myosin, desmin, or myoglobin expression.

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Principles of Cytogenetics

INTRODUCTION

Cancer is a genetic disease characterized by DNA changes at either the nucleotide or chromosomal level, or both. Malignancies can develop either from a genetic predisposition followed by acquired somatic mutations, or from an accumulation of somatic mutations that develop into a cancer phenotype. At the chromosome level, these mutations include changes in chromosome numbers (aneuploidy), loss of heterozygosity (LOH, whole chromosome or segmental region loss), chromosomal rearrangements (translocations and inversions), and gene duplications or amplifications. Many of these cytogenetically visible or cryptic (submicroscopic) aberrations are characteristic of a particular disease or disease subtype. Because specific chromosomal aberrations, especially in hematologic malignancies, provide diagnostic, prognostic, and/or treatment information for many cancers, they are, in many ways, true biomarkers for human cancer.

Clear insights into the genetic basis of cancer were obtained in the 1950s and 1960s when improved cell culture and slide preparation techniques made it possible to accurately enumerate the number of human chromosomes as 46 in 1956 [1]. Chromosome analysis is usually carried out on cells in mitosis (cell division) when the chromosomes become visible as distinct entities. After identifying each chromosome in a cell by its characteristic size, shape, and staining properties, a karyotype displaying the full chromosome complement of the cell can be prepared.

The first specific chromosome abnormality observed in a human tumor was seen in Philadelphia in 1960 by Nowell and Hungerford [2] who found an unusually small

chromosome in the leukemic cells of patients with chronic myeloid leukemia (CML). This small chromosome was named the “Philadelphia” (*Ph*) chromosome. The discovery of the *Ph* chromosome aroused considerable interest in cancer cytogenetics as it gave the first direct evidence for a consistent DNA-associated change in a tumor. More than 30,000 cases of hematologic malignancies with chromosome aberrations have been reported, thereby making it the most thoroughly cytogenetically investigated group of all neoplastic disorders.

A second major breakthrough in cytogenetics was the development of microscopic staining techniques, generating a banding pattern along the length of the chromosomes [3]. With this banding pattern, all individual chromosomes could be identified and structural changes could be characterized in much greater detail. Consistent chromosome aberrations, which are uncommon or extremely rare in normal tissues, were found in different cells of a tumor and further karyotypic changes were shown to occur during tumor progression. Modifications of culture methods to improve yields of dividing cells and high-resolution banding of elongated chromosomes now allow for a more precise definition of rearrangements as well as the identification of previously undetected rearrangements. By using these techniques, most tumor cells can be shown to have some form of chromosomal defect. Possibly, the best correlation between the presence of highly specific chromosomal changes and a subtype of leukemia is the 15;17 translocation which is identified only in patients with acute promyelocytic leukemia (APL or AML-M3). Because the chromosomal aberrations determined at diagnosis is an independent prognosis, indicator in several leukemias and lymphomas,

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it is imperative that the karyotype analyses are completed. As a result of relative ease of obtaining bone marrow or peripheral blood specimens from leukemia patients, it is possible to do serial sampling, which allows for studying the cytogenetic patterns during the various stages of the clinical course, such as at diagnosis, remission, and relapse. This requires appropriate preparations and culturing of the bone marrow. A number of techniques are available to evaluate chromosomal aberrations in hematological malignancies.

CELL PREPARATION

Bone marrow cells from leukemia patients are an ideal source of tissue for cytogenetic studies of leukemias. The use of “direct” preparation to avoid selection during culturing has been considered advantageous for obtaining an accurate picture of the chromosomal constitution of the leukemic cells. However, it has also been suggested that culturing leukemic bone marrow cells may uncover both clonal abnormalities and a greater number of abnormal cells than the direct method of preparation. Chromosome analysis of leukemic cells is not always possible by the direct method because cases are often encountered in which only a few or no mitoses are present or whose chromosomes show blurred outlines and do not provide good banding patterns that are adequate for detailed analysis despite good mitotic index. It must also be noted that the 15;17 translocation characteristic of APL is usually not observed in the direct preparations but easily seen in 24-h cultured bone marrow cells. Thus, it is important that more than one kind of culture setup and harvest method be performed.

A prerequisite for chromosome analysis is dividing or mitotic cells. Spontaneously dividing cells suitable for direct chromosome preparations are found only in the rapidly proliferating tissues of the body such as the gonads and bone marrow, or in tissues with malignancies. Bone marrow is the tissue of choice for cytogenetic study of most hematological conditions. However, in chronic disorders where there is high white cell count, such as CML-blast crisis and chronic lymphocytic leukemia (CLL), a hypercellular peripheral blood sample is more appropriate. It is also important to recognize that even a very dilute sample can overgrow and subsequently fail. Spleen tissue, or more rarely ascitic and pleural effusions, is also amenable for cytogenetic study in some hematological disorders.

Chromosome studies of malignant lymphomas are usually based on studies from lymph node biopsies because bone marrow may not always be involved in the early stages of the disease. But occasionally, a bone marrow may be taken which can prove informative particularly if there is doubt as to whether or not the bone marrow involvement has occurred. The success of a cytogenetic analysis mostly depends on the quality of material investigated. Therefore, the key to successful cytogenetics is adequate sampling with a high viability. The sample must be drawn under sterile conditions with the aspiration syringe coated with preservative-free heparin to avoid clumping of blood components. Heparin can also be added to lymph node and spleen tissue

after their surgical removal. Where there is a likelihood of a dry tap, especially in diseases such as primary or secondary myelofibrosis or due to faulty technique, a peripheral blood sample can be sent as an alternative. However, unless there are sufficient blast cells in these samples, the abnormal clone may go undetected. Care must also be taken in suspected APL cases where clotting is possible and may cause the sample to be unsuitable for culture and chromosome analysis.

A good quality bone marrow aspirate or bone core biopsy sample of 1–2 mL is adequate in most cases, although less than this could be accepted if the marrow is very cellular. The drawing of tissue must be done under sterile conditions because the chromosome analysis is, in most cases, preceded by short- or long-term cell culture (96 h) which mandates a high degree of sterility. It is also advisable to determine if the initial aspirate has bone marrow spicules. If a previous aspirate has been obtained, aspiration of a second sample after repositioning is recommended for cytogenetic studies.

The shorter the duration of time (≤ 24 h) between collection and culture setup in the cytogenetic laboratory, the greater the chances for a successful chromosome analysis with an accurate result. Every effort must be made to ensure that the bone marrow or lymph node biopsy samples are set up in cultures with a minimum delay. If a delay in the transportation of the sample is anticipated, the sample should preferably be collected and transferred to a tube containing transportation medium made up of a preservative-free heparin in an appropriate basal medium and supplemented with serum and antibiotics. The sample must never be frozen but can be stored at 4°C overnight or for up to 3 days. However, the cell viability is greatly reduced with time, yielding misleading or only normal results. Disorders such as acute lymphoblastic leukemia (ALL) and others with a high white cell count are particularly adversely affected by delays.

Same day or direct cultures are often recommended for ALL, and sometimes for CML. Studies suggest that erythropoietic cells divide rapidly in the first few hours of culture followed by granulopoietic cell divisions [4, 5]. On the basis of these observations, short cultures in erythroleukemia are more likely to yield good results. However, in a majority of cases a minimum of 16–24 h unstimulated culture is appropriate. On the other hand, CLL and some ALL cases, which have B- or T-cell phenotype, need 3–5 day cultures with appropriate mitogens, as well as having some unstimulated cultures. Sometimes, due to poor response to the commonly used mitogens, (e.g. chronic B- and T-lymphoid leukemias), TPA (12-O-tetradecanoylphorbol-13-acetate), and EBV (Epstein-Barr virus), pokeweed or IL-2 are used as stimulants in the cultures. Several laboratories supplement the regular media with a condition medium derived from cultures of a human urinary bladder carcinoma or giant cell tumor (GCT) cell lines, which are capable of stimulating the proliferation and growth of human myeloid leukemia cells.

The chromosomes of patients with ALL are particularly difficult, fuzzy, and resistant to banding. Nevertheless, analysis of direct preparation of these marrows have shown that 50–78% of these patients have chromosomal abnormalities in their leukemic cells. But it is important to recognize that more than one technique is necessary to assess accurately the karyotypic constitution of the leukemic cells.

BANDING TECHNIQUES

The standard cytogenetics method consists of culturing a suspension of cells in mitogenic media for 24–72 h. Then, the dividing cells are arrested in metaphase by the addition of an inhibitor of the mitotic spindle, such as colchicine or vinblastine. The cells are submitted to a hypotonic solution (commonly 0.075M KCl) and stained with Giemsa (G-banding), which reveals characteristic banding patterns that are specific for each chromosome. These banding patterns allow the assignment of homologous chromosomes, the identification of extra or missing chromosomes as well as of structural aberrations. For G-banding patterns, pre-treatment of chromosomes by enzymes such as trypsin or pancreatin is required. The mechanism of the G-banding is not fully understood yet but the chromosomes express dark and light G-bands. It is the most common and traditional banding technique used in the clinical setting. Other chromosome banding techniques are used to produce a reverse banding pattern (R-bands), a fluorescence banding technique using quinacrine derivatives (Q-bands), or centromeric staining (C-bands) to better define the chromosomal aberrations. The nucleolus organizer regions (NORs) located in the short arms of acrocentric chromosomes are visualized by staining with silver nitrate (AgNO_3).

ANALYSIS

Chromosome analysis is performed using a microscope commonly at 1000 \times magnification. With the development of image analysis hardware and software, computer-aided chromosome analysis systems are in use and have greatly reduced the turnaround times, and have also made the quality of the

karyotyped images almost equal to that of photographed ones. Bone marrow karyotype analysis is often biased toward cells with poor morphology where there is a mixed population. Selection of only metaphases with good morphology can often lead to failure of detecting the cells from abnormal clones. A clone is defined as at least two cells with the same structural abnormality or gain of the same chromosome or at least three cells with the loss of the same chromosome. The karyotype results are interpreted using an International System for Human Cytogenetic Nomenclature (ISCN 2005, [6]). For example, a normal male and female karyotypes are designated as 46,XY and 46,XX, respectively. An abnormal karyotype such as 46,XY,t(9;22)(q34;q11.2) designates an abnormal male karyotype with a balanced translocation between chromosomes 9 and 22 at band 34 of the long arm of chromosome 9 and the long arm of chromosome 22 at band 11.2. In contrast, a karyotype 47,XY,t(9;22)(q34;q11.2), +der(22)t(9;22) delineates an abnormal male karyotype not only with a balanced 9;22 translocation as explained earlier, but also an additional chromosome that is derived from this translocation, i.e. an extra *P*_h chromosome. The number of cells observed for a particular clone is provided in brackets []. For example, a karyotype 47,XX,+8[12]/46,XX[8], indicates that of the total 20 metaphase cells analyzed from this female patient, 12 cells forming the abnormal clone, had trisomy 8 and the second clone of 8 cells had a normal karyotype.

There are three main types of cytogenetic aberrations in human cancer:

1. Balanced chromosomal rearrangements (translocations or inversions) (Figures 3.1 and 3.2).
2. Gain or loss of whole chromosomes (aneuploidy), or part of a chromosome (segmental aneuploidy) (Figures 3.3 and 3.4).
3. Loss of heterozygosity (LOH).



FIGURE 3.1 An abnormal female karyotype showing a balanced 11;14 translocation and trisomy 12. The karyotype is designated as 47,XX,t(11;14)(q13;q32), +12.

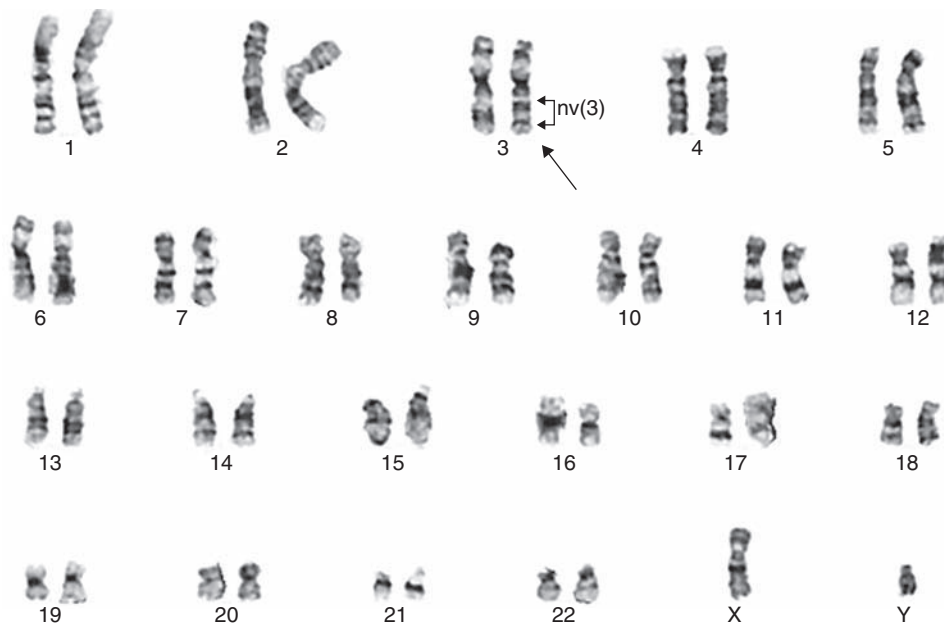


FIGURE 3.2 An abnormal male karyotype with a paracentric inversion in the long arm of one chromosome 3; the region of inversion is shown in brackets. The karyotype is 46,XY,inv(3)(q21q26).

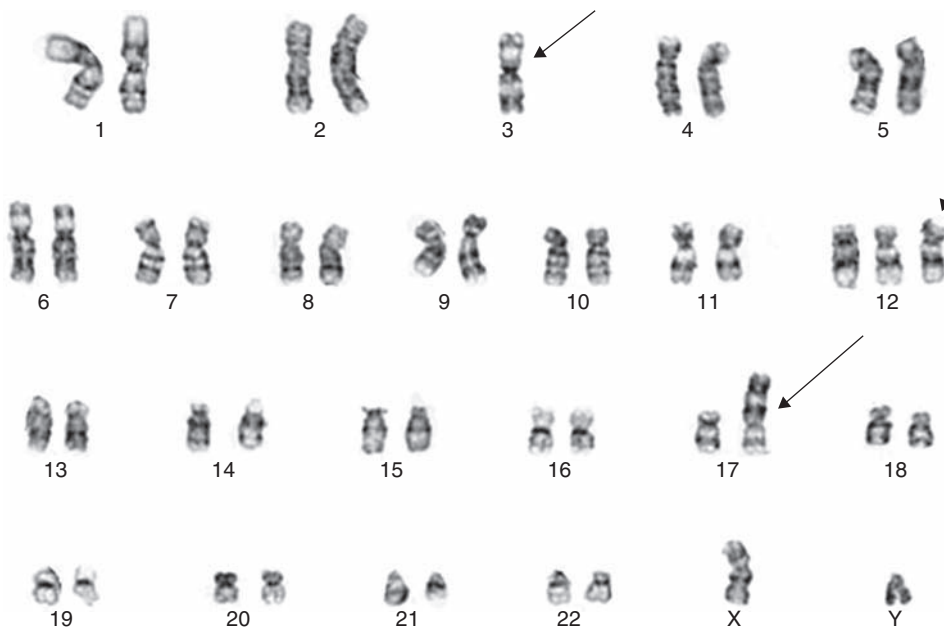


FIGURE 3.3 An abnormal male karyotype with monosomy of chromosome 3, trisomy 12, and an unbalanced translocation between the long arm of chromosome 3 and the short arm of chromosome 17 resulting in the deletion of short arm of chromosome 3 and distal 17p segment. The karyotype is designated as 46,XY, +12,der(17)t(3;17)(q13;p13).

These three types of chromosomal aberrations typically cause cell overgrowth through over-expression/activation of an oncogene, or by deletion of a tumor suppressor gene. Identification of recurrent chromosomal aberrations has become very important in the diagnosis of soft tissue and hematologic tumors. Especially in some hematologic malignancies, the identification of recurrent chromosomal aberrations is important for diagnosis, classification, prognosis, and therapy.

Balanced Rearrangements

Balanced rearrangements in cancer include translocations (exchange between two or more chromosomes) and inversions (orientation change relative to the centromere, within a single chromosome/arm). These rearrangements often result in chimeric cellular proteins that appear to disrupt the normal function of critical genes involved in normal cell growth or differentiation resulting in an abnormal process. More than

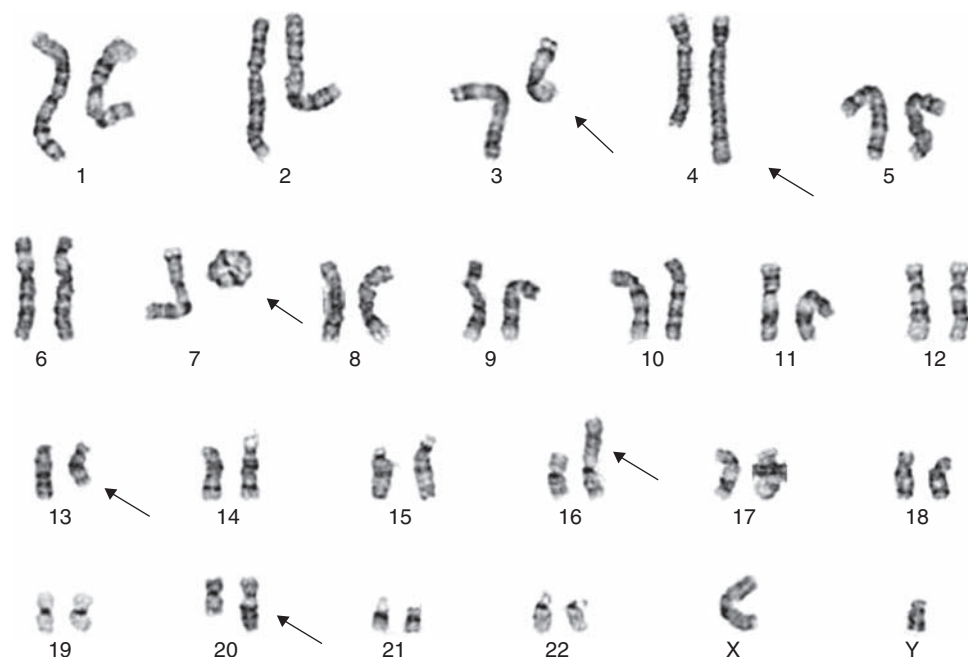


FIGURE 3.4 An abnormal complex male karyotype with several aberrations including a deletion of 3q, an interstitial deletion of 13q, and an unbalanced translocation with unidentifiable chromosomal segments of chromosomes 4q, 16p, and 20q, respectively, and ring chromosome 7. The karyotype is written as 46,XY,del(3)(q21),add(4)(q35),r(7),del(13)(q12q14),add(16)(p13),add(20)(q13).

600 neoplasia-related recurrent balanced cytogenetic aberrations have been reported to date [7]. Translocations and inversions usually cause cancer by fusing together two genes, resulting in aberrant expression. Currently, more than two hundred fusion genes responsible for human cancers have been reported in the literature [8, 9]. One classic example of an important translocation in human cancer is the t(9;22) in CML. The t(9;22) results in aberrant expression of a gene (*ABL1*) that normally functions in cellular proliferation by coming under control of a constitutively expressed gene (*BCR*). Approximately 50% of hematopoietic neoplasms acquire translocations somatically; most of these neoplasms are restricted to a single cell lineage (that in which the translocation originated) and are arrested in a particular stage of developmental maturation. Occasionally, more than one cell lineage is affected (e.g. *MLL* gene-related malignancies) suggesting that the involved genes were affected at the pluripotent stem cell stage. While balanced aberrations may be directly related to the etiology of the malignancy, the unbalanced translocations are often recognized as indicators of secondary tumor progression.

Chromosomal Aneuploidy

Chromosomal aneuploidy is extremely common in cancer, and can be either a primary or a secondary event [10]. Chromosomal gains (whole or partial) are designated in the karyotype with “+” or “add,” and typically result in the over-expression of an oncogene. Despite the presence of a high frequency of aneuploidy in cancer, the exact role of aneuploidy in carcinogenesis is not very clear. Numerical

aberrations as the sole karyotypic anomalies, including single or multiple losses and gains, are found in approximately 15% of all cytogenetically abnormal hematologic neoplasms (Figure 3.5) [8]. Although they are relatively frequent, numerical abnormalities have generally received less attention than the structural abnormalities, particularly the simple reciprocal translocations that are amenable to rigorous molecular analysis. The association of numerical aberrations with hematologic disorders, although well established, also appears less disease specific [11–14]. Trisomy 8, monosomy 7, and trisomy 21 have been found in different categories of leukemias both at initial presentation and as secondary cytogenetic events. Roughly, half of all numerical aberrations are trisomies. Other than trisomies for chromosomes 8, 9, 11, 12, and 21, autosomal trisomies are infrequent in hematologic disorders. Aneuploidy can be detected with the help of traditional metaphase cytogenetics (Figure 3.5), interphase cytogenetics (fluorescence *in situ* hybridization (FISH) (Figure 3.6), multicolor FISH, spectral karyotyping, comparative genomic hybridization techniques (CGH)), flow cytometry (FCM), and image cytometry (ICM). Flow cytometry and ICM can measure the relative DNA content of the cell with respect to reference diploid cells. Imbalances, i.e. aberrations that result in gain or loss of genetic material, are even more common than translocations and inversions in hematologic malignancies. These include amplifications, duplications, heterozygous or homozygous deletions, monosomies, and trisomies. Amplifications (several extra copies of a gene or chromosome region) may occur in the form of supernumerary marker chromosomes (SMCs), double minutes, and homologous staining regions (HSRs); and are a result of over-expression of one or more genes (Figure 3.6c).

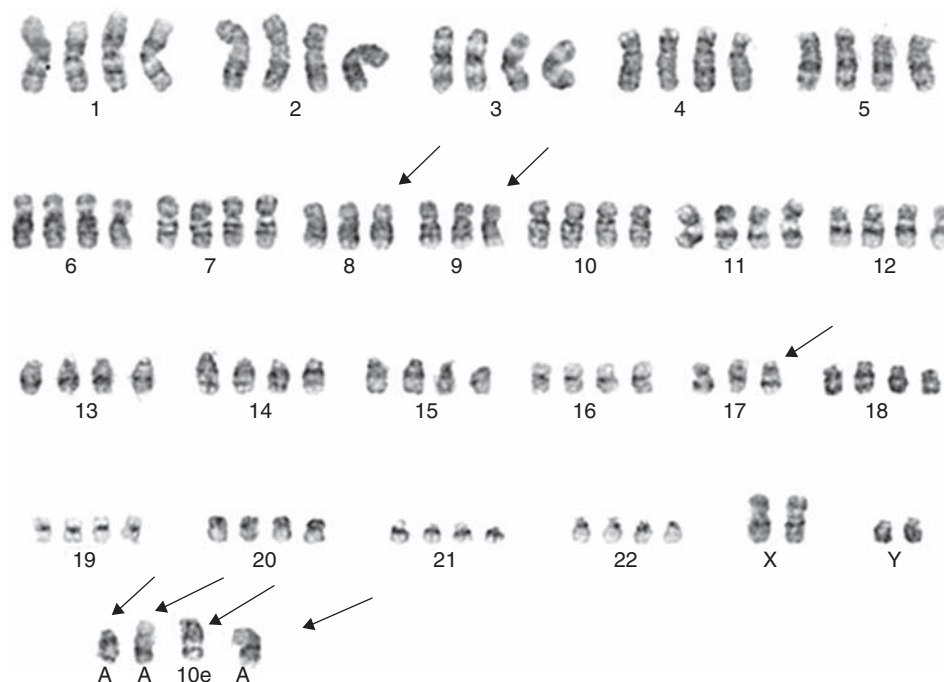


FIGURE 3.5 An abnormal male hyperdiploid (near-tetraploid) karyotype with four copies of all autosomes except for chromosomes 8, 9, 17, and extra copies of small marker chromosomes of unknown origin. This karyotype is written as 93,XY, +X, +Y, -8, -9, -17, +4mar.

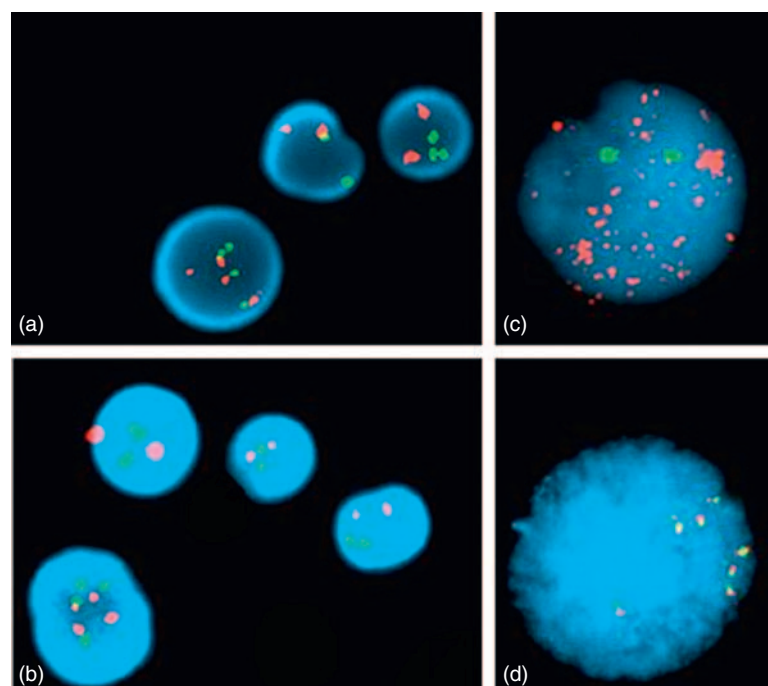


FIGURE 3.6 Panel showing identification of various chromosomal abnormalities by FISH. (a) two–four copies of the 11q13 (red) and 14q32 (green) loci, (b) four copies of 9q34 (red) and 22q11.2 (green) loci, (c) amplification of the *ABL* oncogene (red), and normal two copies of the *BCR* (green) locus, and (d) six copies of the *MLL* locus (yellow).

SMCs are small additional chromosomes whose origins are not readily identifiable by banding methodologies, and are designated as “+mar” in the karyotype (see Figure 3.5). Double minutes are specific types of SMCs that are characterized by a typical dumbbell shape and represent extra-chromosomal oncogene amplification. For example,

MYCN gene amplification in the form of double minutes is commonly observed in neuroblastoma. The mixed lineage leukemia gene (*MLL*) is sometimes amplified in acute leukemia and can be easily visualized in interphase nuclei by FISH studies with *MLL*-specific probes (Figure 3.6d). HSRs are amplified oncogenes within the structure of a

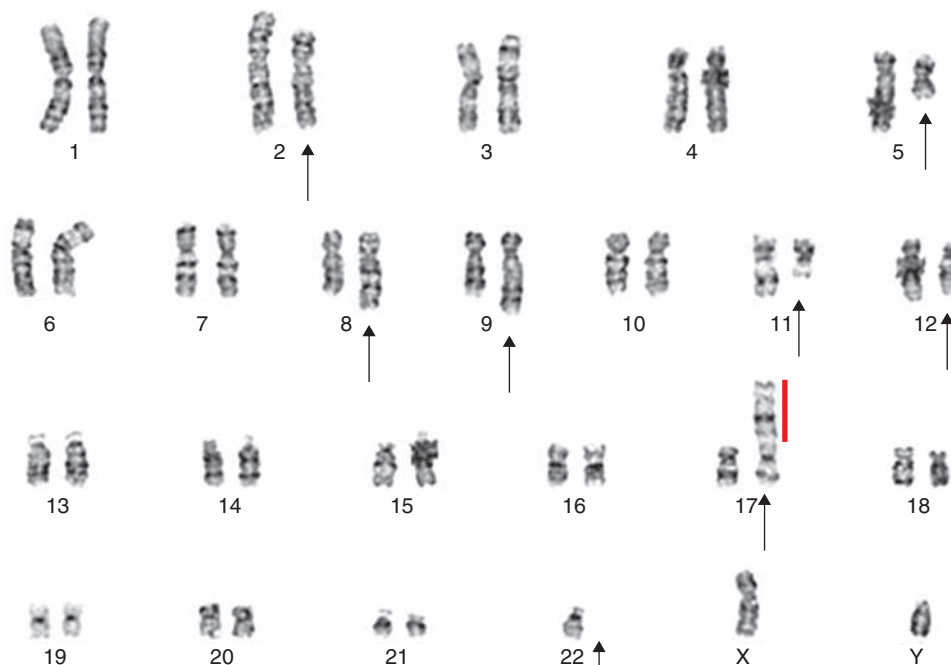


FIGURE 3.7 An abnormal male karyotype with an HSR of an unknown chromosomal region at the short arm of chromosome 17 (red line).

chromosome (Figure 3.7), and are designated as “HSR” in the karyotype. Usually *C-MYC* gene amplification on chromosome 8 in some acute myeloid leukemias (AMLs) is an example of an HSR that is typically detected with FISH. Chromosomal losses (whole or partial) are designated in the karyotype by “-” or “del,” and are thought to result in deletion (or decreased activity) of tumor suppressor genes.

Loss of Heterozygosity

Loss of heterozygosity is defined as the loss of one parent’s contribution to the cell and can be caused by deletion, gene conversion, mitotic recombination, or loss of a chromosome. LOH often occurs in cancer, where the second copy of a gene (typically a tumor suppressor gene) has been inactivated by other mechanisms, such as point mutation or hypermethylation. When a whole chromosome or a large segment of a chromosome is lost, the remaining chromosome or segment is often duplicated. With complete duplication of the remaining genetic material, the karyotype may appear normal, even though no normal genes are present. Though not easily detected by cytogenetic techniques, this duplication of the remaining chromosome or segment has been shown using molecular genetic techniques [5, 15]. At least in theory this type of LOH can be detected cytogenetically using chromosome heteromorphisms, though it is not often pursued.

Cytogenetic analyses can provide valuable and extremely relevant information to establish the presence of a malignant clone, determine the cell lineages in the disease process or clarify and confirm a diagnosis, provide prognostic predictive features, and monitor response to treatment and classification of neoplasms. The significance and usefulness of these will be discussed in the following chapters.

A close relationship between the pathologist and the cytogeneticist is essential if maximum useful information is to be produced from the cytogenetic studies of hematological disorders.

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Principles of Molecular Techniques

FLUORESCENCE *IN SITU* HYBRIDIZATION

Karyotype analysis depends primarily on classical chromosomal banding techniques; and has the distinct advantage that the entire genome can be analyzed in a single experiment. In particular, it is useful for identifying whole chromosomes accurately and for identifying obvious chromosomal aberrations. However, karyotype studies are limited to actively dividing cells, and the resolution is limited to chromosomal rearrangements that are >3 Mb in size. In addition, though there is a history of several decades of clinical cytogenetic analysis of cancer cells, it has become apparent that suboptimal collection, transport, and culture of clinical specimens can lead to inappropriate (e.g. normal) results. Poorly spread or contracted metaphase chromosomes, low mitotic activity, and highly rearranged karyotypes with numerous marker chromosomes, common in neoplastic cell preparations, are often difficult to interpret unambiguously. Furthermore, chromosome preparations are labor-intensive and time-consuming and the interpretation of cytogenetic findings require extensive experience. Although automated karyotyping systems became available, analyzing metaphase spreads remains time-consuming. Techniques such as polymerase chain reaction (PCR) have the advantage to be more sensitive and to screen for a specific chromosome aberration without the need for dividing cells. However, such molecular analyses are limited to known fusion genes and do not allow for the screening of the whole genome for other (secondary) alterations. Thus molecular cytogenetic techniques have been developed to bridge the gap between classical cytogenetics and molecular

DNA techniques. The limitations of classical chromosome studies have been overcome by the introduction of fluorescence *in situ* hybridization (FISH), which offers a molecular dimension to cytogenetic analysis. Different and new FISH technologies have emerged, each with their own particular advantages and applications, e.g. interphase FISH, comparative genomic hybridization (CGH), fiber-FISH, and multi-color FISH. These techniques are capable of detecting aberrations of an intermediate size (~ 10 kb to 5 Mb), and are commonly used in cancer cytogenetics laboratories today, for both diagnostic and research applications. These techniques are fast and provide an accurate but targeted analysis of whole tumor genomes in a single experiment. The FISH technologies provide increased resolution for the elucidation of structural chromosome abnormalities that cannot be resolved by more conventional cytogenetic analyses, including submicroscopic deletions, cryptic or subtle duplications and translocations, complex rearrangements involving many chromosomes, and marker chromosomes.

The FISH procedure has been developed for the tagging of DNA and RNA with labeled nucleic acid probes and is a process whereby chromosomes or portions of chromosomes are vividly painted with fluorescent molecules that anneal to specific regions. This technique has been used widely for the identification of chromosomal abnormalities. The method enables enumeration of multiple copies of chromosomes or detection of specific regions of DNA or RNA that represent associations with certain genetic characteristics and infectious disease.

The FISH methods widely employed in clinical laboratory studies involve hybridization of a fluorochrome-labeled DNA probe to an *in situ* chromosomal target and can be applied to a

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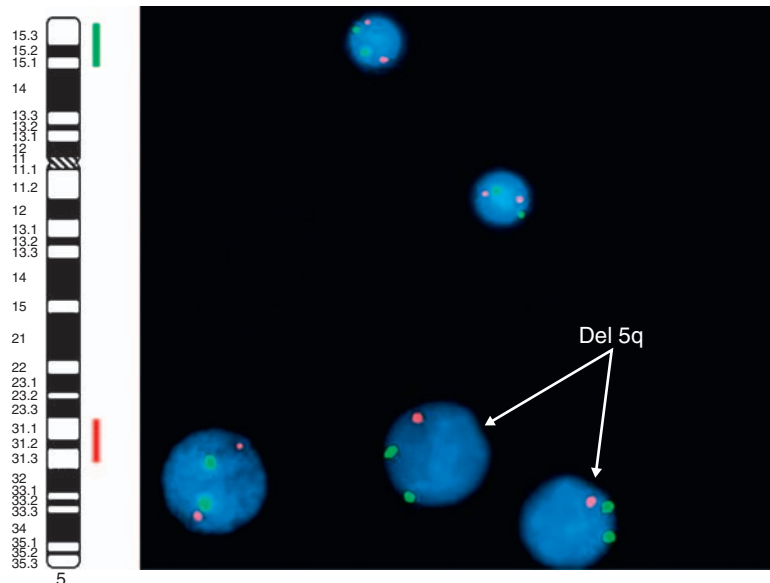


FIGURE 4.1 Deletion of 5q detected by 5p (green) (control) and 5q (red) specific FISH probes.

variety of specimen types and performed on nondividing interphase cells. Interphase nucleus assessment from uncultured preparations allows for a rapid screening for specific chromosome rearrangements or numerical abnormalities associated with hematologic malignancies. Interphase analysis may also be performed on fixed bone marrow cell suspensions, paraffin-embedded tissue sections or disaggregated cells from paraffin blocks, bone marrow or blood smears, and touch preparations of cells from lymph nodes or solid tumors. It is also commonly used when rapid or direct (i.e. without culturing) results are needed and can be performed on formalin-fixed paraffin-embedded (FFPE) tissue. FISH uses fluorescently labeled DNA probes (e.g. bacterial artificial chromosomes, or BACs) hybridized to either metaphase chromosomes or interphase nuclei, depending on the application. However analytically powerful and diagnostically useful interphase FISH might be, great care should be taken in the interpretation of interphase hybridization patterns. As a rule of thumb, FISH results should be interpreted in conjunction with the neoplastic karyotype.

Four different types of probes are commonly used, each with different ranges of applications:

1. Gene-specific probes target DNA sequences (Figure 4.1) present in only one copy per chromosome. They are used to identify chromosomal translocations, inversions and deletions, contiguous gene syndromes, and chromosomal amplifications in interphase and metaphase chromosomes. These probes are particularly useful for screening of specific chromosomal aberrations in metaphase spreads and interphase nuclei. For this purpose, the probes cover the chromosomal breakpoints and can specifically identify the genes involved in the chromosome alterations without the need for dividing cells. The same FISH experiments can subsequently be used to assess the efficacy of therapeutic regimens and to detect residual disease with a rather limited sensitivity of 0.5–5%.

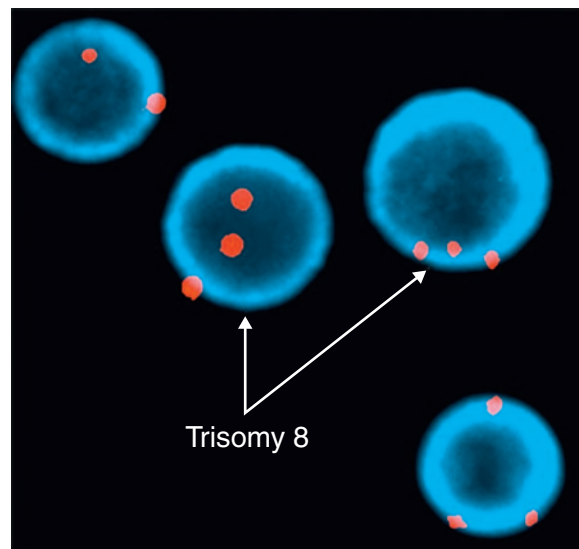


FIGURE 4.2 FISH studies reveal three copies of chromosome 8 with centromere specific probe in each cell (arrows), indicative of trisomy 8.

2. Repetitive sequence probes (Figure 4.2) (alpha-satellite sequences) bind to chromosomal regions that are represented by short repetitive base-pair sequences that are present in multiple copies (e.g. centromeric and telomeric probes). Centromeres are usually A–T rich, whereas telomeres are known to have repetitive TTAGGG sequences. Centromeric probes are extremely useful for identifying marker chromosomes and for detecting copy number chromosome abnormalities in interphase nuclei.
3. Subtelomeric probes (Figure 4.3) are frequently used to identify subtle or submicroscopic chromosomal rearrangements. The relative ease of performance and high resolution (0.5 Mb) of these unique sequence have made them popular to screen for chromosomal

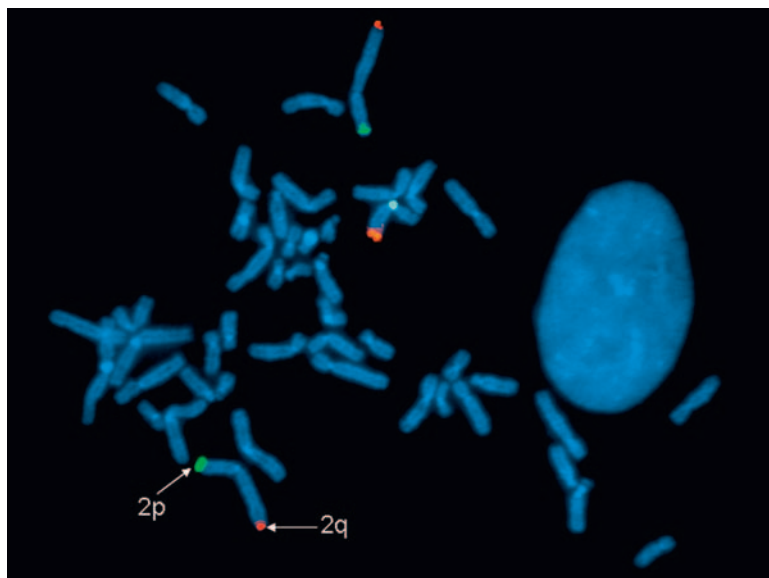


FIGURE 4.3 Subtelomere specific FISH probes are demonstrated in chromosomes 2 and Xp, respectively.

rearrangement probes, subtelomeric deletions, and marker chromosomes.

4. Whole-chromosome painting (WCP) probes (Figure 4.4) are complex DNA probes that are generated by degenerate oligonucleotide polymerase chain reaction or through flow sorting. WCP probes have high affinity for the whole chromosome along its entire length, with the exception of the centromeric and telomeric regions. These probes are most suitable for identifying genomic imbalances in metaphase chromosomes, especially the complex chromosomal arrangements observed in many cancers. Two variants of WCP probes have been developed, multi-color FISH (M-FISH) and spectral karyotyping (SKY). WCP probes have been developed that paint all human chromosomes in different colors (48 paints). And WCP probes can also offer simultaneous detection of each arm of all human chromosomes in a single hybridization. This technique is usually used in conjunction with chromosomal banding techniques for a more precise identification of chromosome aberrations. The two greatest limitations of WCP probes are: (1) extensive knowledge of genetic abnormality to enable the correct selection of the probes and (2) limited resolution to detect chromosomal inversions and very small deletions/amplifications and translocations due to its limited resolution of >2–3 Mb.

Three main probe strategies are utilized in FISH: (1) enumerating probes, (2) fusion probes, and (3) “break-apart” probes.

Enumerating Probe

The *enumerating*, or counting, probe strategy, as its name implies, is useful for counting the number of a particular

locus or whole chromosomes within the cell. Counting probes are used to detect gains or losses of whole chromosomes (e.g. chromosomes 5, 7, 8, and 20 in myelodysplastic syndrome, MDS) (Figures 4.5 and 4.6) or deletions and duplications of genes involved in a disease (e.g. *TP53* and *RB1* gene probes in myeloma). These probes can be either BACs containing the gene or genes of interest, or alpha-satellite repeat sequences specific for the centromeric region of each chromosome. This strategy is also useful for detecting cryptic deletions that cannot be detected by classical metaphase chromosome analysis.

Fusion Probe

The fusion probe strategy is classically used to detect translocations or inversions [e.g. the t(9;22) in chronic myeloid leukemia, CML (Figures 4.7 and 4.9) and the t(15;17) in APL]. BAC probes complementary to chromosomal regions involved in the rearrangements are labeled with two different fluorophores (e.g. red and green) and analyzed under the microscope for signal overlap. Normal nuclei will have two red and two green signals, corresponding to the two normal (un-rearranged) chromosomes, while nuclei with rearrangements will have one or more yellow signals, corresponding to the overlap of the red and green signals and suggestive of fused chromosomes. Dual-fusion strategies are used to reduce false-positive signals produced by artifactual overlap caused by the three-dimensional structure of DNA compaction within the nucleus. Dual-fusion approaches utilize probes that overlap the two reciprocal translocation breakpoints and result in two yellow fusion signals corresponding to the two derivative chromosomes. This probe strategy is also useful to distinguish between variants, such as an extra Philadelphia chromosome in CML blast crisis.

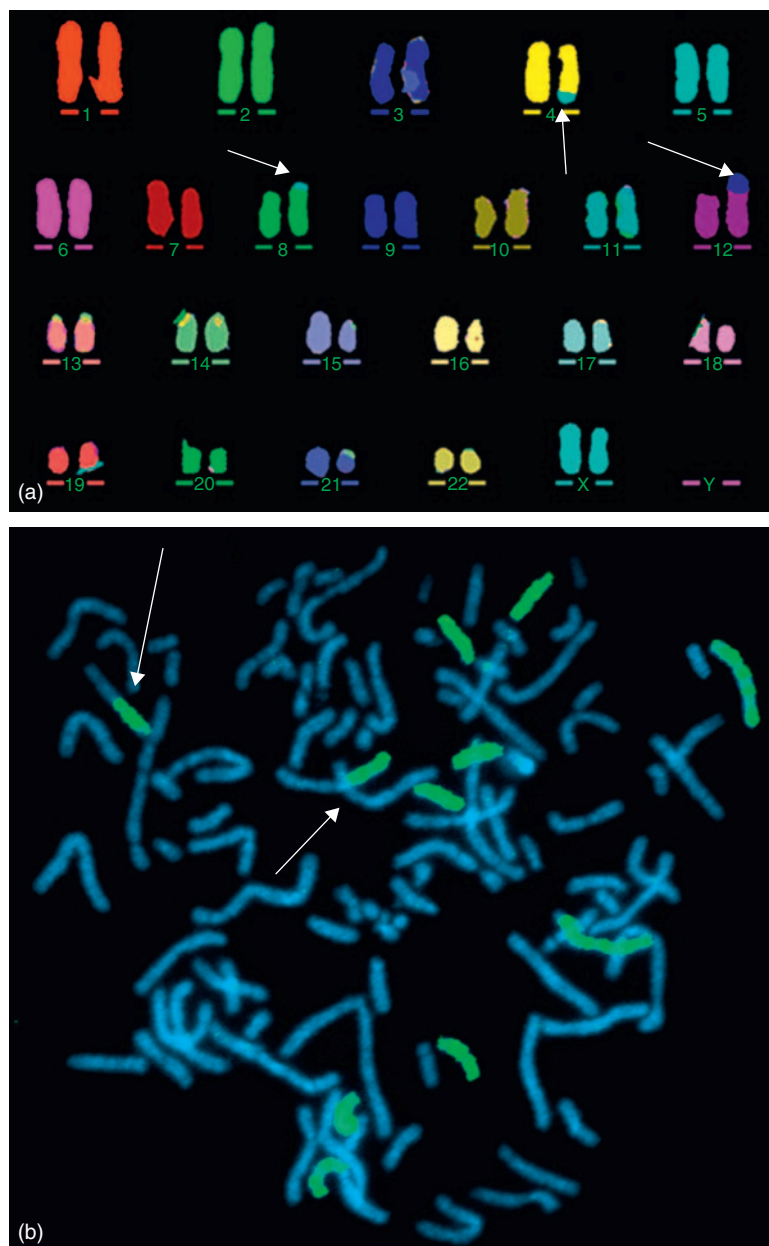


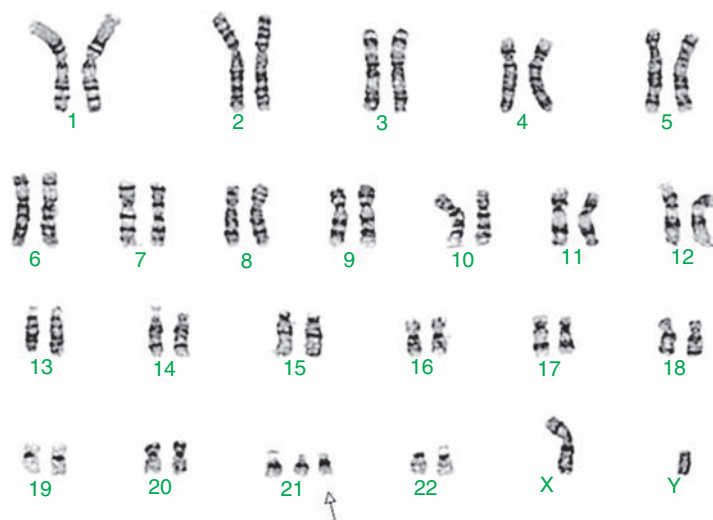
FIGURE 4.4 Chromosome painting. Translocations (arrows) are demonstrated by multi-color FISH technique (a) and chromosome 7 is highlighted with whole chromosome paint (b).

Break-Apart Probe

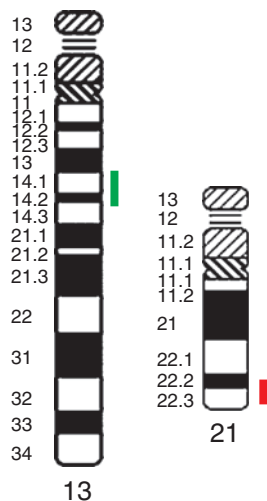
The break-apart probe strategy (Figure 4.8) is essentially the opposite of the fusion probe strategy and is most useful when a single locus is involved in several different rearrangements (translocations, inversions, deletions, etc.) involving multiple partners. For example, a dual-color FISH probe has been developed with probes on either side of the *MLL* gene breakpoint, resulting in separation of the normally co-localizing signals if the *MLL* gene is rearranged. The advantage of this system is that it can detect all recurrent and possibly novel *MLL* rearrangements in a single experiment. The *MLL* gene locus is involved in >70 recurrent

translocations [1], all of which can be detected with the break-apart strategy. Two differently labeled BAC probes (e.g. red and green) normally bind to a single locus and produce the overlapped signal color (e.g. yellow). When the locus of interest is rearranged, the colors split apart. Normal nuclei will have two yellow (overlapped) signals, while nuclei with a rearrangement will have one yellow, one red, and one green signals.

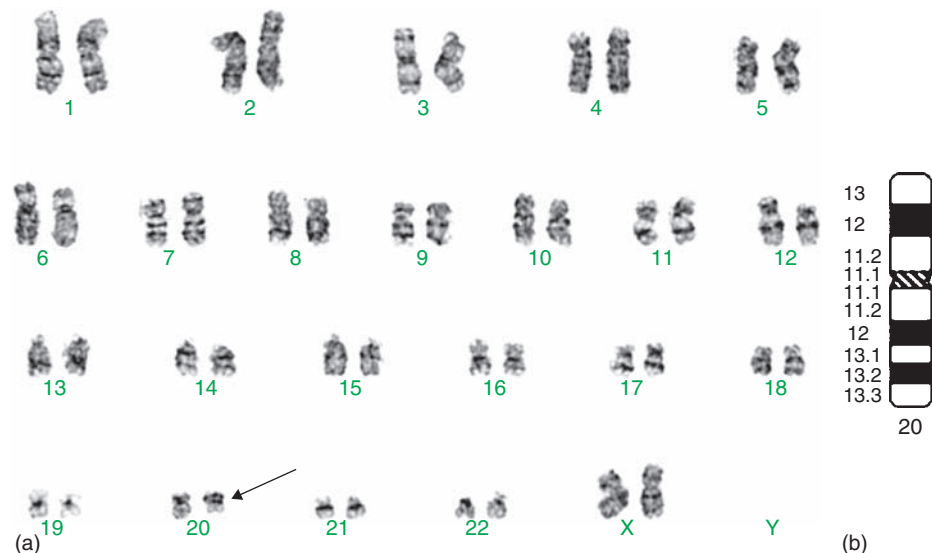
While FISH can provide rapid results and is applicable for various sample types that are otherwise not amenable to classic cytogenetic analyses, it has limitations. FISH will only answer the particular question being asked regarding an exact probed locus. For example, cells probed with a



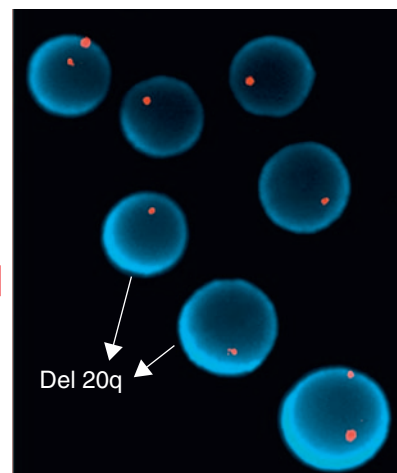
(a)



(b)

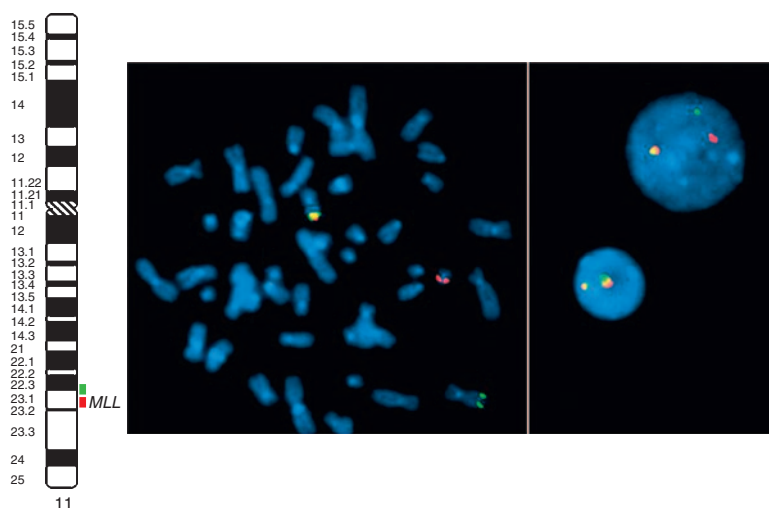
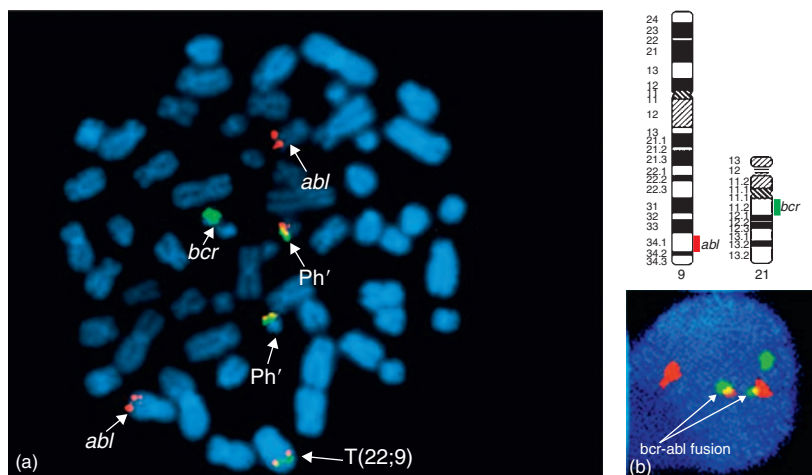


(a)



(b)

FIGURE 4.6 Deletion of chromosome 20q, 46,XX,del(20)(q11.2) is demonstrated by G-banded karyotyping (a) and FISH (b, arrows).



BCR-ABL1 fusion probe set may be positive for the t(9;22), but trisomy 8 cells within the sample (often seen in CML-blast crisis) would not be detected unless a chromosome 8 enumerating probe set is used in the probe mix. Similarly, an enumerating probe set consisting only of the alpha repeats from the chromosome 5 centromere will not detect a deletion of the long arm of chromosome 5 (5q-). Physicians ordering tests should be mindful of the questions they are trying to address and order FISH and/or karyotypes appropriately. FISH studies may also be an integral component of the diagnostic work-up if a specific genetic abnormality is suggested by histopathology, peripheral blood counts, or clinical parameters, or when cytogenetic analysis fails or provides a normal karyotypic result. For example, it is recommended that all cases of CML be studied at diagnosis by cytogenetic analysis and molecular cytogenetic methods to determine the initial clonal abnormalities and the FISH signal pattern both for prognostic information and for follow-up studies [2]. When questions arise regarding which FISH test to order, the laboratory should be consulted, as ordering a FISH test without specifying the probe set is inadequate. The application of FISH further provides increased sensitivity, in that chromosomal abnormalities have been

detected in samples that appeared to be normal by conventional cytogenetic analysis, e.g. a chromosomally hidden or unidentifiable translocation has been evidenced for the first time in 1995 with the discovery of the t(12;21)(p13;q22), resulting in ETV6/AML1 fusion (Figure 4.9). Molecular cytogenetic investigations with probes specific for this gene rearrangement revealed the translocation to be present in 25% of all pediatric B-ALL [3].

The comparative genomic hybridization (CGH) technique is a relatively new molecular technique for identifying gains and losses in a test sample (e.g. a patient sample), relative to a control sample. DNA is extracted from both the test and control samples and digested with restriction enzymes or sonicated to break it into short (~ 500 bp) fragments. The test and control samples are differentially labeled with fluorescent dyes (e.g. red and green), denatured, and hybridized to metaphase chromosomes. Chromosomal regions that are equally represented in the test and control samples will hybridize equally to the chromosomes and produce an overlapped color (e.g. yellow). A loss (deletion) is detected when the control DNA fluoresces stronger within a region of the metaphase chromosomes, and a gain (duplication) is detected when the patient DNA fluoresces stronger.

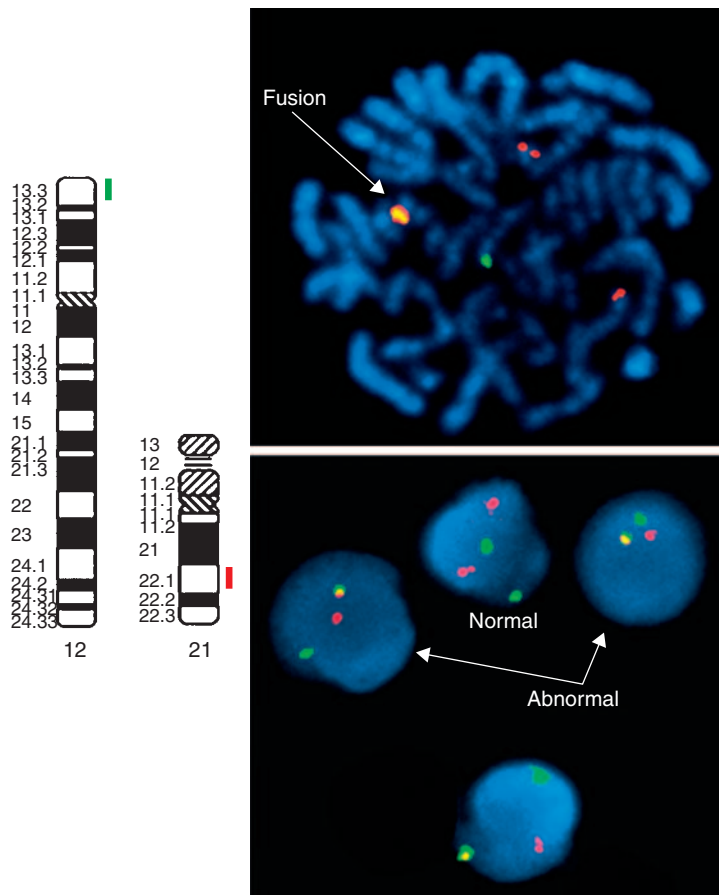


FIGURE 4.9 Demonstration of t(12;21): The fusion of *ETV6* (red) and *RUNX1* (green) genes demonstrates a yellow spot by dual-color FISH.

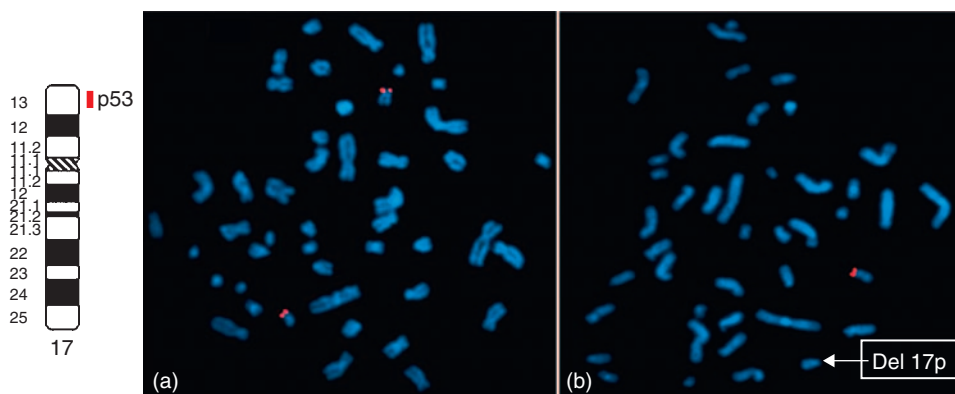


FIGURE 4.10 A normal metaphase with p53 FISH probe reveals normal 17p (two red signals (a)) and (b) a metaphase with deletion of 17p.

In array CGH (aCGH), the fluorescently labeled test and control samples are hybridized to an array of DNA sequences [e.g. BACs (Figure 4.11) or oligonucleotides (Figure 4.12)] rather than metaphase chromosomes. Array CGH has a much higher resolution than classic metaphase CGH. Although CGH and aCGH have been well established for use in detecting submicroscopic gains and losses in constitutional (inherited) disease [4], neither is currently appropriately established as a stand-alone technology for diagnosis

in cancer. One reason is that the CGH technique cannot detect balanced chromosomal aberrations (translocations and inversions), which are very common in cancers, especially in hematological disorders. Also, because of tumor heterogeneity and general view of genomic instability, i.e. several clonal populations, CGH and aCGH do not necessarily provide a narrow and consistent genomic regions of interest that can be definitely implicated or identify previously unknown genomic regions of primary etiology. However, with improvements

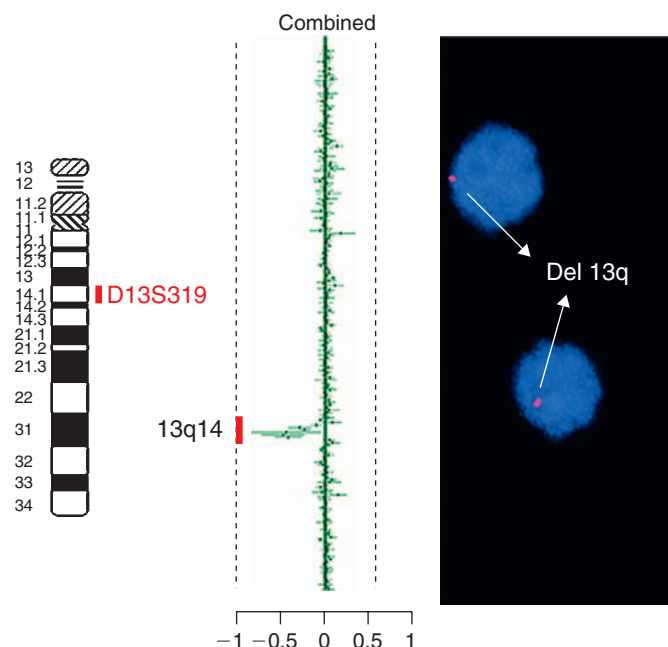


FIGURE 4.11 A whole-genome BAC-array CGH showing a deletion of 13q.

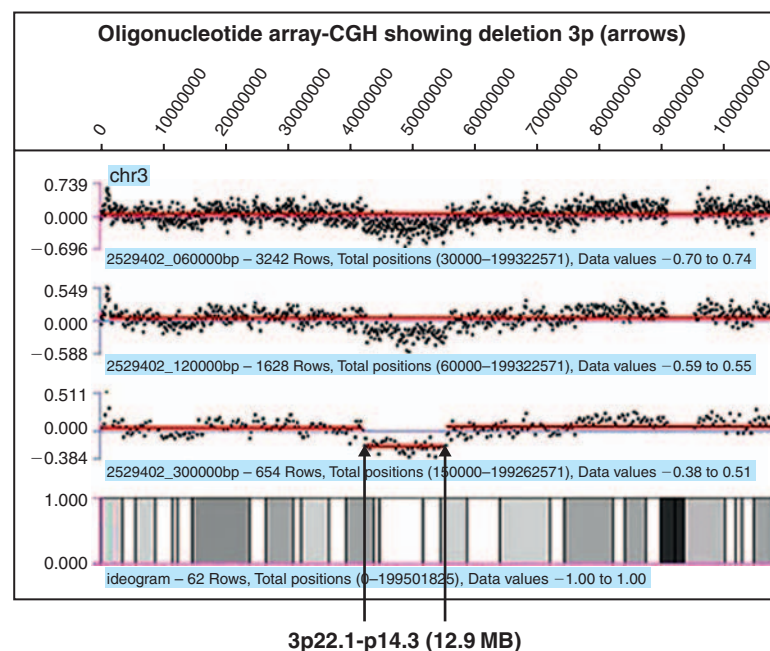


FIGURE 4.12 Shift at 42150000...55050000 bp; chromosome 3p22-p14.3 (12.9 MB).

in technology, software analyses and database collection, CGH will be one of the most useful techniques adopted by diagnostic laboratories [5]. Indeed, it would be interesting to see if aCGH enhances the efficiency of detecting subtle changes such as partial tandem duplication/amplification of the *MLL* gene observed in acute myeloid leukemia (AML). Furthermore, in chronic diseases such as MDS or CML, where a progressively evolving karyotype relates to worsening prognosis that may be used in therapeutic decisions, aCGH

would provide very useful comparative genome-wide information in sequential clinical specimens.

Fluorescence *in situ* hybridization is also a useful tool to monitor remission status when clonal chromosome abnormalities have been identified at diagnosis and appropriate probes are available. For CML, sequential FISH studies are particularly useful to determine changes in clinical status in response to therapy and to assess for minimal residual disease. In patients with sex-mismatched bone marrow

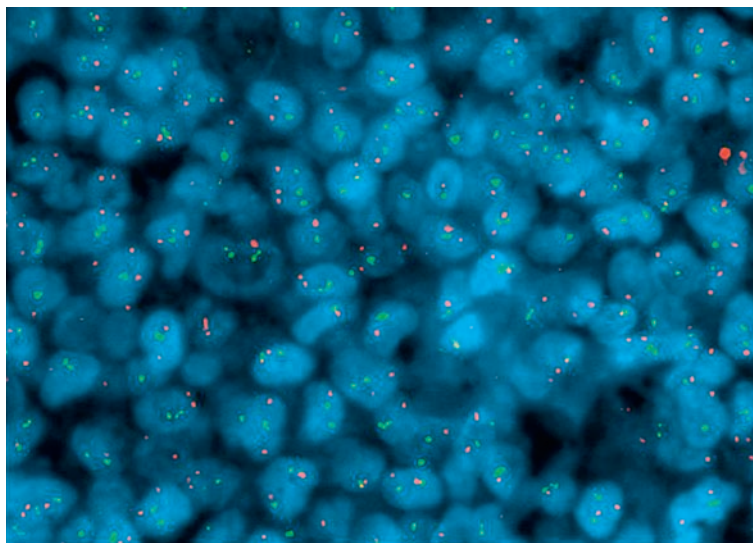


FIGURE 4.13 A typical image of FISH analysis on FFPE section showing overlapping truncated cells.

transplants for whom graft rejection, marrow suppression, or disease relapse is a clinical consideration, monitoring with a FISH assay that combines sex chromosome probes with or without probes to detect the patient's clonal abnormality can be valuable for graft assessment and for detecting residual or recurrent disease.

Fluorescence *in situ* hybridization may be performed on FFPE sections by hybridizing directly either to unstained thin sections (2–4 microns) of tissue that have been deparaffinized or to thick sections of tissue from which individual cell suspensions are made, to which standard FISH techniques may then be applied. Sections must be mounted on slides that will reduce the loss of tissue during FISH-pretreatment processes. Fixation in buffered formalin (pH 7.0) is the best fixative for FISH hybridization. However, fixation in solutions that contain a heavy metal or picric acid often results in unsuccessful FISH results. There are several limitations to FFPE FISH, including overlapping and truncated cells, making assessment of individual cells difficult (Figure 4.13). Also, improvements in the pretreatment, hybridization, optimal probe size, and postwash techniques will alleviate the commonly encountered problems such as background (nonspecific binding) or absence of probe signals. FISH on frozen-tissue sections will yield good results if the slides are first slowly thawed at room temperature and then fixed in 10% buffered formalin.

There are only a few commercially manufactured probe kits that have been approved by the Food and Drug Administration (FDA) for *in vitro* diagnostic testing. These FISH kits must meet the sensitivity and specificity parameters stated in package inserts provided by the manufacturer. The majority of probes used for clinical FISH testing are considered Analyte Specific Reagents (ASRs) that are exempt from FDA approval. When a new ASR probe is introduced in the laboratory, extensive validation is needed, including specific validation of the probe itself (*probe validation*) and validation of the procedures utilizing the probe (*analytical validation*) [6, 7]. Initially, it is important to become familiar with a probe's parameters including signal intensity and pattern and any cross-hybridization that is likely to confound test results. Probe sensitivity, defined as the

percentage of metaphases with the expected signal pattern at the correct chromosomal location, should be established. Likewise, probe specificity, defined as the percentage of signals that hybridize to the correct locus and no other location, must also be assessed. Probes used for hematologic malignancy studies should have a high analytic sensitivity and specificity (>95%), particularly if they are to be used for minimal residual disease assessment.

Fluorescence *in situ* hybridization results should be interpreted within the broader context of probe and analytical validation [7]. The interpretation of FISH results should include consideration of the reason for referral for testing and, when available, additional laboratory findings including conventional cytogenetic analysis, hematopathology, and immunophenotyping [8]. When acute promyelocytic leukemia (APL) is the suspected diagnosis, FISH should be performed on a STAT basis with 24–48 h turnaround time to allow for timely treatment with all-trans-retinoic acid (ATRA) [9].

A system for FISH nomenclature, including both metaphase and interphase analysis, has been developed [10]. While the system may seem confusing to those not working directly with chromosomes, correct nomenclature designations are important to convey the precise nature of a result. For example, metaphase FISH ISCN (International Society of Chromosome Nomenclature) for a male patient with a 9;22 translocation resulting in fusion of the *BCR* and *ABL1* genes studied with conventional banding and with a dual-color, single-fusion *BCR/ABL1* probe set would be written: 46,XY,t(9;22)(q34;q11.2).isht(9;22)(*ABL1*-,*BCR*+,*ABL1*+) indicating that the probe sequence from the *ABL1* locus is missing from the derivative chromosome 9 and is present on the derivative chromosome 22 distal to the *BCR* locus.

The same rearrangement expressed in interphase FISH nomenclature but using a dual-color, dual-fusion probe set would be: nuc ish 9q34(*ABL1* × 3), 22q11.2(*BCR* × 3) (*ABL1* con *BCR* × 2), indicating that each of the probes has been split apart and juxtaposed by the translocation. The use of such precise ISCN is valued by laboratories in the initial diagnostic work-up and continued monitoring of patients with a specific chromosome abnormality. The report

must indicate any specific limitations of the assay, some of which may be described in the probe manufacture's package insert.

While interphase FISH analysis provides information only on specific probes used and generally does not substitute for complete karyotype analysis, it may, under some disease circumstances, be the preferred means of identifying an abnormal clone, e.g. FISH with the *ATM*, *CEP12*, *D13S319*, and *TP53* probe panel in B-cell chronic lymphocytic leukemia (CLL) [11], discrimination of the inversion 16 in AML (M4-Eo) [12], or FISH for the diagnostic abnormality in post-therapy patients who have hypocellular marrows.

Fluorescence *in situ* hybridization has now become an invaluable tool in defining and monitoring acquired chromosome abnormalities associated with hematologic and other neoplasias. The implementation of the technology into the routine diagnostic laboratory requires rigorous attention to when it is appropriate to apply the technology, a very systematic approach to the validation of probes and technical procedures involved in FISH and training of individuals who will perform the testing, and a comprehensive, but plain and simple, means of reporting out results. As the number of critical loci involved in neoplastic chromosome rearrangements or numeric abnormalities continues to expand, the diversity of FISH probes and unique probe sets will undoubtedly increase. FISH has become an important means both for the definition of the initial chromosome changes in a disease process and a reliable means for the ongoing monitoring of response to therapy and disease remission.

Apart from the diagnostic approaches, FISH can be used as a research tool to refine the breakpoint regions of novel chromosome abnormalities, which is often an essential step in the identification of new (partner) genes involved in leukemogenesis. Recent progress of the Human Genome Project has facilitated the characterization of the translocation breakpoints using FISH. PAC/BAC resources, covering the entire genome are available and can be easily found using the databases of the University of California, Santa Cruz and the National Center for Biotechnology Information (<http://genome.ucsc.edu/>, <http://www.ensembl.org/>, <http://www.ncbi.nlm.nih.gov/genome/guide/human/>). These clones can be used to determine more precisely the breakpoint regions and to search for genes involved in the translocations.

The brief overview of the genetic tools and strategies described earlier have been applied to cancer for fewer than 50 years, but they have quickly been recognized to be invaluable in the study and diagnosis of malignancy. The importance of cytogenetics in oncology is evidenced by the reclassification of certain hematological diseases by the World Health Organization, and the application of both classical and molecular cytogenetic methods to hematologic diseases will be presented throughout this book.

PCR AND RELATED TECHNIQUES

It is impossible to overstate the degree to which the advent of nucleic acid amplification techniques, especially the PCR,

has revolutionized the molecular approaches to hematopathologic diagnosis and molecular diagnostics generally. PCR and reverse transcriptase PCR (RT-PCR) not only enable robust analysis of scant or degraded specimens, but also allow for precise quantitation of the analyte, fine dissection of a particular locus or sequence from among the over 3 billion nucleotides of the human genome, and access to otherwise difficult specimens such as FFPE tissue biopsies. These techniques have largely replaced the much more laborious and time-consuming Southern blot (discussed later) for most, but not all, applications in hematopathology. At a more basic level, they have also replaced difficult DNA-cloning procedures for many purposes, including the generation of DNA probes used in a variety of downstream applications such as the Southern blot. On the other hand, they have a number of limitations and technical pitfalls, one of which is that the sequence of portions of the target gene must be known, which is not necessarily the case for certain cloning experiments.

Basic Technique

Polymerase chain reaction is so simple in its conception that it is surprising no one in molecular biology happened to think of it before the seminal paper by Saiki *et al.* appeared in 1988 [13]. Fundamentally it merely mimics replication of DNA *in vivo*, using essentially the same enzymes (DNA polymerases), but in a highly specific and exponential fashion. The specificity results from the use of specific *primer* sequences that are constructed to be complementary only to the target gene or region of interest. The exponential amplification results from the use of a pair of primers which flank the target region and hybridize to opposite strands of the DNA (Figure 4.14). Serving as start-sites for the polymerase reaction, they promote a bidirectional synthesis of daughter strands that then become templates for the next round of replication. The replication cycles are controlled by alternate heating and cooling of the sample, denaturing the amplification products and then enabling the primers (which are present in great excess) to re-hybridize and begin the replication again. Since each replication cycle doubles the number of template molecules, the products accumulate in exponential, rather than linear, fashion. A typical experiment encompasses about 30 cycles, performed in a programmable heating/cooling instrument called a *thermal cycler*, which produces amplification of many millionfold. The precise timing and temperature settings will vary depending on the target sequence and application but generally fall in the range of about 94°C for denaturation and 50–60°C for renaturation. The elongation step, in which the actual DNA synthesis occurs, is run at 70–75°C. The reason it is not performed at physiologic temperature is that in modern PCR the DNA polymerases used are cloned from a variety of thermophilic microorganisms (e.g. *Thermus aquaticus*, source of so-called *Taq polymerase*) so that they do not degrade during each denaturation step [14].

Primer Design

For PCR to deliver the specificity desired, it is most important that primer sequences be chosen carefully so they do

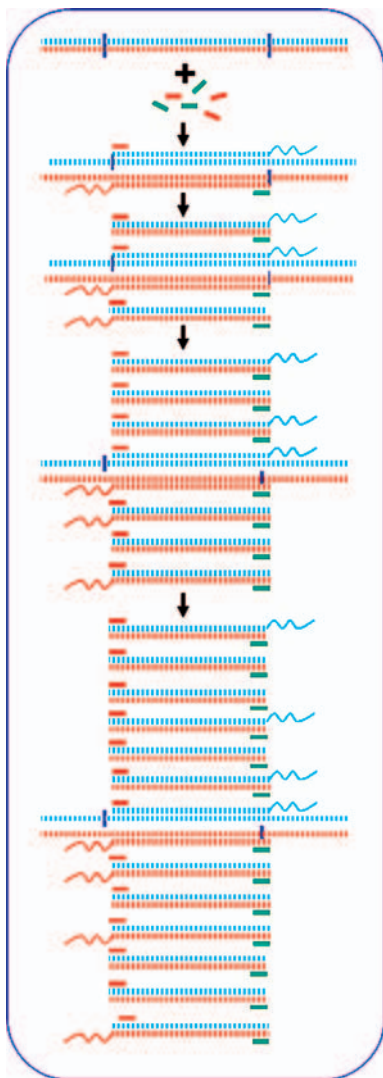


FIGURE 4.14 Polymerase chain reaction scheme. The oligonucleotide primers (rectangles) are designed to hybridize to opposite strands some distance apart on the target region of interest. When hybridized, they serve as priming sites for DNA polymerase replication of each strand. Alternate heating and cooling allows for multiple cycles of denaturation, rehybridization, and replication, increasing the amount of the target sequence exponentially.

not cross-hybridize with other regions of the genome at the renaturation temperatures used. Primers are typically about 18–25 nucleotides in length, and there are computer programs available on the Internet to work out optimal base composition such that the primers do not hybridize elsewhere or to themselves. For most clinical purposes, primers are designed to hybridize a few hundred nucleotides apart in the target sequence, since replication efficiency declines as target length increases. A number of long-range PCR protocols and commercial kits are available, allowing for amplification of targets many thousands of base pairs in length [15], but these are mostly reserved for research purposes.

Quality Control

Given the awesome sensitivity of PCR, theoretically down to a single-target DNA molecule [16], extreme care must be exercised to guard against the production of spurious amplification products by contaminant target molecules. In a clinical laboratory, these can come from other patient specimens, from the operator himself, or from residual amplification products of a previous assay. In practice, it is this last source that is the most concerning, since these products are present in infinitely greater excess than stray genomic contaminants from an individual specimen. A large number of precautionary and preventative procedures have been developed to guard against it, including one-way workflow from pre- to post-amplification areas, special dedicated pipets, re-gloving, and degradation of residual amplicons by ultraviolet light and other methods. Failing this, contamination is detected by the running of a “no DNA” or “no template” control tube in every PCR assay. This tube contains all the requisite enzymes and nucleotides for the reaction to proceed, but no target template to copy. If any amplicon is observed in this sample after PCR, it indicates a contamination problem with that run or with one of the reagents. In addition, known positive controls must be run with each assay, to demonstrate capability of the PCR conditions and reagents to detect and amplify the desired target sequence when present.

Product Analysis

For most applications, PCR is not an end in itself but rather a first step in a subsequent assay. Once the desired target has been amplified, it may be analyzed by hybridization with specific DNA probes in a dot blot format, by size fractionation in agarose gel or capillary electrophoresis, by digestion with sequence-specific restriction endonucleases, or by DNA sequencing. The choice of downstream technique will depend on the nature of the disease process being tested, as will become clear from the specific clinical examples presented throughout this book.

Reverse Transcriptases PCR

Certain disease applications, for example the detection of the *BCR-ABL* translocation in CML (see Chapter 7), involve detection and accurate quantification of an RNA, rather than a DNA, target. Since PCR uses *DNA* polymerases which do not replicate or transcribe RNA, amplification of such targets can only be accomplished by first converting them to DNA. Fortunately, there are viral-derived enzymes available that can do this, termed *reverse transcriptases* (RT). Thus, amplification and study of RNA targets utilizes the technique of RT-PCR, in which the first step is reverse transcription, followed then by conventional PCR of the resulting DNA products.

Real-Time PCR

As noted earlier, in most cases PCR amplification is the first step in an assay that then utilizes another method to

analyze or quantify the products. This is because conventional thermal cyclers are closed systems in which amplification proceeds without observation until it is finished and the reaction tubes removed. In contrast, a newer generation of thermal cycler instruments can measure the accumulation of amplicon as it occurs (i.e. in real time) by measuring the incorporation of a fluorescent label into the elongating products. These instruments can provide very precise quantification of products and, by extrapolation, the amount of starting target material, in the sample. They are very useful for cancer applications in hematopathology designed to detect minimal residual disease after therapy.

Related Amplification Techniques

A large number of innovations to the basic PCR technique have been developed over the years to address particular applications or to circumvent certain pitfalls. Included are such techniques as nested PCR, whole-genome amplification, inverse PCR, hot-start PCR, and allele-specific PCR. For the most part they are beyond the scope of this chapter but will be mentioned in the context of particular disease applications where relevant elsewhere in the book. In addition, a number of non-PCR amplification techniques have been developed over the years, such as Q β replicase, and ligate chain reaction, but for the most part they have fallen by the wayside in favor of PCR, at least for applications relevant to hematopathology (some are used in molecular microbiology and genetics testing).

BLOTTING TECHNIQUES

In blotting techniques, unique segments of nucleic acid sequences (DNA probes) are used to demonstrate the presence of complementary sequence of DNA or RNA in the sample. Since the complementary target is composed of hundreds or thousands of nucleotide bases, the reaction of

the DNA probe to the target (hybridization) is the tightest and most specific intermolecular interaction [17].

Southern Blot

In this technique the DNA probes are labeled with a radioactive or nonradioactive signal moiety. The DNA target is treated with restriction enzymes (endonucleases), and the DNA fragments are separated by gel electrophoresis (usually an agarose gel). The DNA is then transferred to a membrane which is a sheet of special blotting paper. The blot is incubated with numerous copies of a single-stranded labeled DNA probe. The probe hybridizes with its complementary DNA sequence within the target sample to form a labeled double-stranded DNA molecule. The radioactively labeled copy of the DNA probe is then detected by autoradiography (Figure 4.15). In nonradioactive DNA labeling procedures, nucleotides (probes) are conjugated with biotin or other protein binders such as digoxigenin. Biotin binds specifically to the protein avidin with a very high affinity. Avidin is a polyvalent protein which can be linked to chromogenic enzymes, fluorescent compounds or electron-dense particles. The advantage of a radiolabeled probe over a non-radiolabeled probe is its higher sensitivity (5- to 10- folds), and its disadvantages are the elongated radiography step, which may extend to several days, radiation hazard, and requirement for special procedures for disposal of the radioactive contaminated wastes [17, 18].

Northern Blot

Northern blot is a technique basically similar to Southern blot, except that it is used to transfer RNA from a gel to a blot instead of DNA [19].

Dot Blot

Dot blot is similar to the other blotting techniques, except that it does not provide information regarding the size of

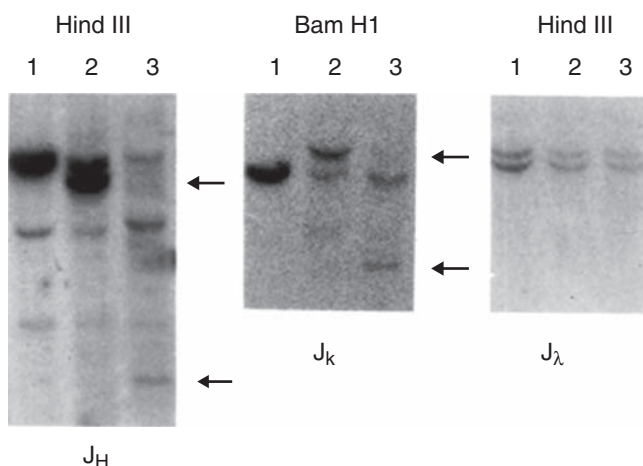


FIGURE 4.15 Southern blot analysis for Ig gene rearrangements demonstrating rearranged bands (arrows) for the joining region of the Ig heavy chain (J_H) and the joining region of the kappa light chain (J_k).

the hybridized fragment. With this technique, extracted DNA or RNA from the target specimen is spotted onto the filter without the prior electrophoresis and transfer steps.

MICROARRAY TECHNIQUES

The next generation of blotting techniques is a marriage of molecular biology and information technology: the microarray. In contrast to a dot blot or Southern blot in which target DNA is hybridized to one or a few probes, microarrays enable the hybridization of hundreds or hundreds of thousands of target sequences simultaneously. Because it is constructed at nanoscale using technology similar to that used to manufacture silicon-based computer chips [20], the vernacular term “DNA chip” is often applied. Essentially it is a reverse dot blot on a grand (and miniaturized) scale. The individual probes, in great numbers, are bound to the solid support, while the specimen DNA (or RNA) is hybridized to them after being labeled with a fluorescent marker (Figure 4.16). A chip-reader instrument interprets these signals and generates data output detailing which sequences hybridized and which did not, as well as the intensity of hybridization (which relates to the relative amount of each particular target sequence in the material being tested).

There are two basic kinds of microarrays: DNA sequence arrays and RNA expression arrays. The former are used to detect the presence of mutations and other sequence variants in the tested sample, whereas the latter are used to assay relative expression of hundreds or thousands of genes, as for example in comparing tumor mRNA to that in a corresponding normal tissue. Depending on how many probes are placed on the array, assays of varying comprehensiveness can be developed, even to the extreme of comprising the entire human genome. Microarrays can even be used for sequencing, if every possible target sequence is encompassed by the probes on the chip.

In a sense, microarrays represent one area where technology has outpaced biological knowledge, at least in the clinical setting. One would be hard pressed to come up with a disease state, in hematopathology or elsewhere, in which our knowledge is so extensive that we need to assay the sequence or expression of 20,000 genes, or even for that matter 200. For this reason, microarray technology remains, at time of this writing, largely in the research sphere, with few accepted clinical applications. However, most people recognize that this is indeed the technology of the future and will find many applications in the clinical laboratory as soon as our knowledge catches up to its technical capabilities. At the same time it must be kept in mind that such comprehensive whole-genome scanning raises a number of ethical issues related to defects and predictive risks that might unwittingly be revealed when applied to patients [21].

DNA SEQUENCING

In many ways DNA sequencing is the most definitive molecular biology technique because it gets directly at the genetic code of the specimen being analyzed, and that is what is at the core of everything else, both biologically and clinically. Other techniques, such as probe hybridization, restriction endonuclease digestion and even PCR, are surrogate assays whose outcome depends ultimately on the inherent sequence of the target material but which do not ascertain that sequence directly. For this reason DNA sequencing is often referred to as the “gold standard” for molecular testing in the clinical setting.

Like other techniques in molecular diagnostics, DNA sequencing arose in the research setting to satisfy research needs and resulted in Nobel Prizes for its inventors. Initially cumbersome, the techniques have been refined over the years, recently under the impetus of the Human Genome Project which demanded extremely-high-throughput, accurate, and inexpensive sequence analysis. The benefits of these

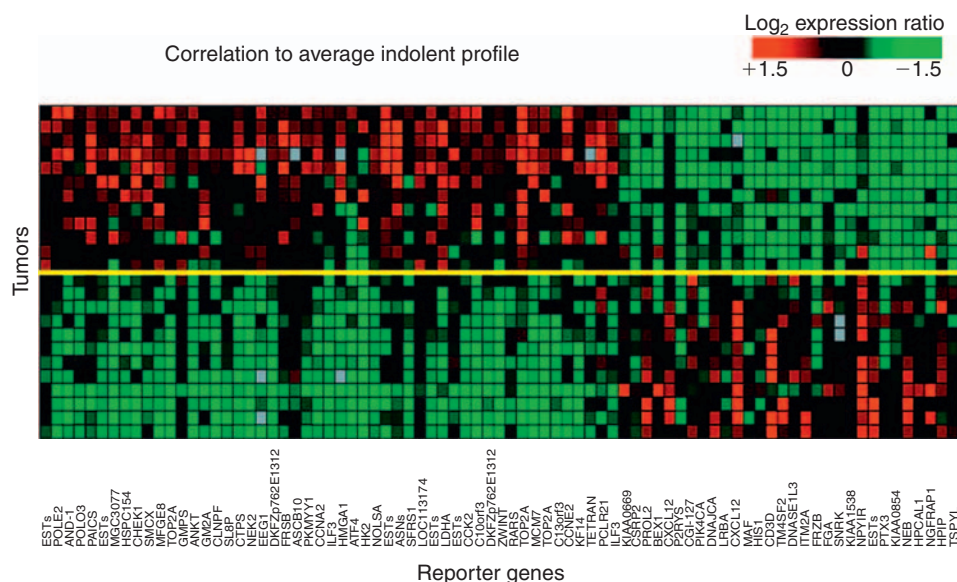


FIGURE 4.16 Gene expression profiling in follicular lymphomas using a microarray technique. Results demonstrate two populations of patients: those with aggressive disease are segregated above the solid yellow line and those with indolent clinical course are placed below it. This research was originally published in *Blood*; Glas AM, Kersten MJ, Delahaye LJ, Witteveen AT, Kibbelaar RE, Velds A, Wessels LF, Joosten P, Kerkhoven RM, Bernards R, van Krieken JH, Kluin PM, van't Veer LJ, de Jong D. (2005). *Blood* **105**, 301–307, by permission.

innovations now spill over into the clinical arena where sequencing, previously considered a kind of “last resort” methodology for special cases, is now routine for a wide variety of applications.

Chemical Methods

Two basic approaches to sequencing were initially developed and used, but one of them has now assumed dominance for both manual and automated platforms. The chemical degradation method, developed by Maxam and Gilbert [22], uses various chemicals to cleave the double helix, based on their reaction with specific nucleotides. Sizing and aligning the resulting fragments by electrophoresis allows one to determine at which point along the length of the helix each nucleotide was positioned. This method has largely been abandoned for routine uses because of its technical difficulty and the noxious nature of the chemicals required.

It has been supplanted by the chain termination method, developed by Sanger *et al.* [23] at about the same time. This one, too, is based on fragment sizes determining the positions of each nucleotide, but the fragments are created not by degradation but by synthesis. Four DNA polymerase reactions are set up using the target sample as the template for replication. In each reaction tube, in addition to the four regular nucleotide substrates, a dideoxynucleotide derivative is also added. When one of these is incorporated into the growing daughter strand by the polymerase, replication

is halted at that point because the dideoxynucleotide lacks the 3'-hydroxyl group required for chemical linkage to the next nucleotide that would otherwise be added. Since the derivative nucleotides are present in the minority, they are not inserted at every spot but rather at a random distribution whenever that nucleotide is required. This produces a complete spread of fragments whose size is based solely on the positions of that particular nucleotide in the original target material. If the fragments from each nucleotide reaction are separated based on size by electrophoresis, a “ladder” is created that reveals in order, from shortest to longest, the nucleotide sequence.

Sequence Detection and Analysis

The electrophoresis can be done in a flat-bed (usually vertical) gel set-up, typically a long polyacrylamide gel, using radioactive nucleotides that can be seen by autoradiography of the dried gel after the run. But more and more clinical laboratories have moved to automated sequencing instruments which use capillary electrophoresis of fluorescently labeled oligonucleotides to generate the sequence. The reactions are carried out in a single tube instead of four, and the various reactions are discriminated because each of the four dideoxynucleotides is labeled with a different-colored fluorophore. These instruments provide more precise sizing, much higher throughput, and sophisticated software for calling out and analyzing the sequence (Figure 4.17).

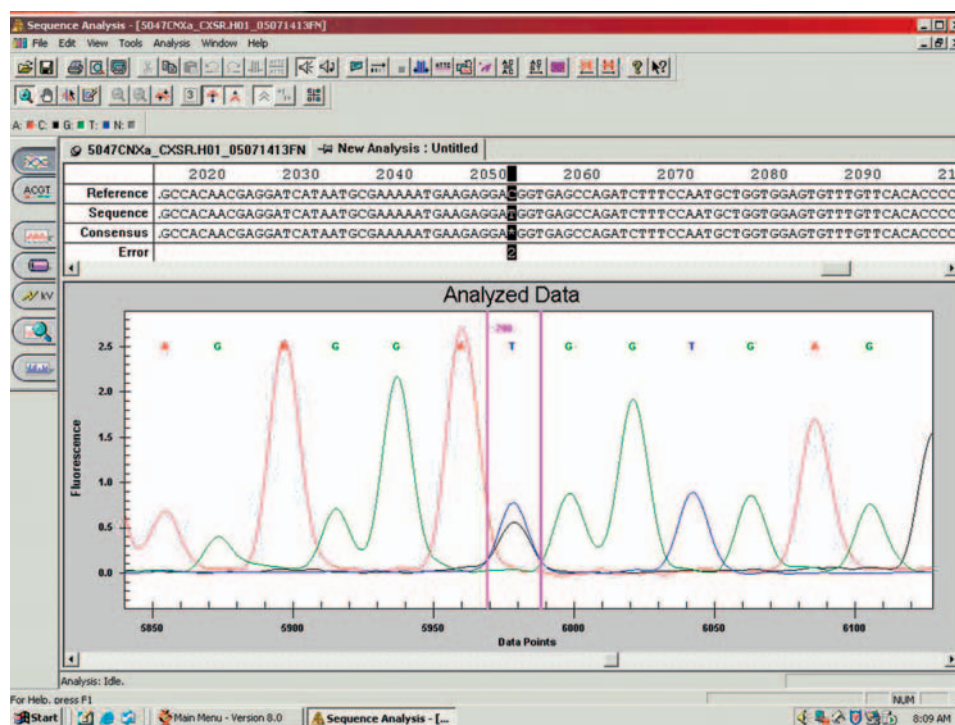


FIGURE 4.17 Example of the read-out from an automated capillary electrophoresis DNA sequencer. Each colored peak represents the capture of a fluorescently labeled DNA fragment by the laser detector of the instrument, in order of size fractionation. Each dideoxynucleotide in the reactions was labeled with a different-colored fluorophore, represented by the colors of the read-out and nucleotide calls by the instrument. Note the heterozygous single-nucleotide substitution (C→T) at position 290 (bracketed by the pink vertical lines).

Limitations of Sequencing

While it may be the “gold standard”, DNA sequencing, like any other technique, has its limitations and pitfalls. For many applications in which only one particular mutation or size fragment is being probed, sequencing may represent “overkill” in that it yields a tremendous amount of extraneous data not relevant to the clinical question being asked. Some of that data, moreover, can be of questionable clinical relevance. Scattered throughout the genome of every human being are countless nonpathologic nucleotide substitutions, called *polymorphisms*. When they occur within protein coding regions (exons), they appear as missense mutations, causing the substitution of one amino acid for another in the gene product. If a sequencing test reveals one of these changes that has not been seen or reported in the literature before, it can be very difficult or impossible to decide whether it represents a pathologic missense mutation or merely a benign polymorphism. Certain physical attributes, such as its position within the protein and the biochemical nature of the amino acid substitution, may help one to deduce its impact, though there are many exceptions to these rules. Correlation with phenotype, presence or absence of the change in other affected or unaffected family members, or the population at large, can also be of help. Ultimately, functional studies of the altered gene *in vitro* may be needed, but these are not applicable to a clinical laboratory.

Aside from the clinical interpretation, even the detection of nucleotide substitutions in the heterozygous state may be problematic. Ironically, this seems to be more of an issue with automated sequencers than with manual systems which depend more on the eye of the operator. A heterozygous substitution will appear on the automated read-out as two differently colored peaks superimposed at the same nucleotide position (see Figure 4.17). For various technical reasons which are difficult to control, the two peaks may not be of the same intensity, even though they are supposedly present in equal amounts in the specimen. If one of the peaks is much smaller than the 20–30% level, it may be ignored by the instrument's software and called out as homozygous for the other nucleotide. One way to guard against missing a heterozygous change in this way is to perform the sequencing in both directions (i.e. using opposite strands of the same fragment as template) and compare the read-outs. It is unlikely that the instrument would miss the change both times.

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Morphology of Abnormal Bone Marrow

This chapter briefly describes morphologic features of bone marrow lesions that will not be covered in the following chapters. These lesions include gelatinous transformation of bone marrow, bone marrow necrosis, amyloidosis, granulomas, metastatic lesions, post-therapeutic changes, and, finally, bone and stromal changes.

GELATINOUS TRANSFORMATION

Gelatinous transformation of bone marrow is characterized by fat atrophy, accumulation of hyaluronic acid (gelatinous material), and bone marrow hypoplasia [1–3]. This condition has been observed in various chronic debilitating conditions such as anorexia nervosa and other forms of severe starvations, as well as in malignancies, tuberculosis, chronic renal failure ulcerative colitis, and AIDS [4–7].

The bone marrow involvement is often patchy, and the gelatinous amorphous, glassy deposit appears as a light blue substance by H&E stain and reacts positively with alcian blue and periodic acid Schiff (PAS) stains (Figure 5.1). Bone marrow smears stained with Wright's stain show a bluish-pink material mixed with adipocytes (Figure 5.1c).

The reversal of gelatinous transformation has been observed in patients with anorexia nervosa when their nutritional status has been improved [8].

BONE MARROW NECROSIS

Extensive bone marrow necrosis is infrequent and is usually associated with hematologic disorders, such as leukemias/lymphomas and sickle cell anemia, bone marrow metastasis, or infections [9–13]. The cause of bone marrow necrosis could be attributed to the following factors [14–19]:

1. Vascular occlusion caused by deformed red cells (sickle cell anemia), fibrin clot (disseminated intravascular coagulation, thrombotic thrombocytopenic purpura), or vascular occlusion by fungi (such as mucormycosis) or tumor emboli.
2. Inadequate blood supply, such as in severe anemia or hyperparathyroidism.
3. Infection, such as gram-positive and gram-negative bacteria, Q fever, typhoid fever, tuberculosis, diphtheria, and histoplasmosis.
4. Radiation and chemotherapy.
5. Release of tumor necrosis factor (TNF) in cancer patients.

Extensive bone marrow necrosis may be associated with extramedullary hematopoiesis or leukoerythroblastosis (presence of immature erythroid and myeloid cells in the peripheral blood) and pancytopenia. The bone marrow biopsy sections show coagulation or fibrinoid necrosis. The coagulation necrosis is more

Faramarz Naeim

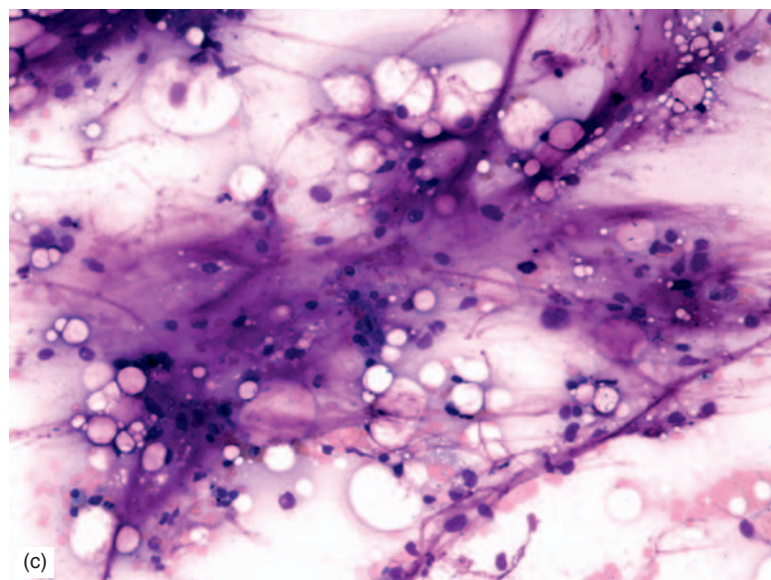
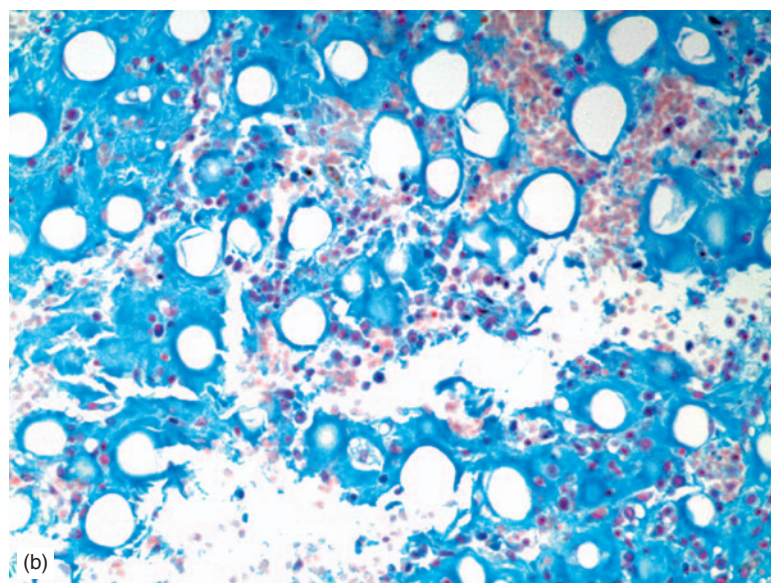
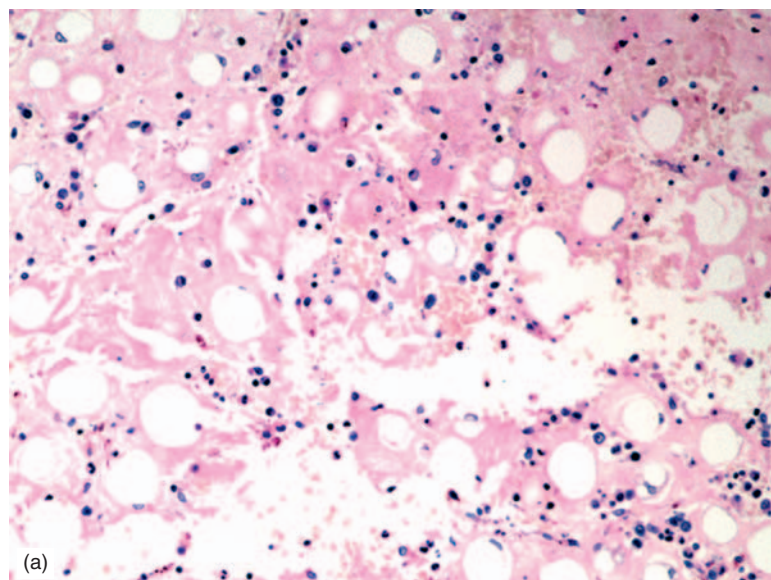


FIGURE 5.1 Gelatinous transformation of fatty tissue: (a) Bone marrow biopsy section demonstrating hypocellularity and partial replacement of the bone marrow fat by an eosinophilic amorphous substance. (b) The substance reacts positively with the alcian blue stain. (c) Bone marrow smear is hypocellular and shows increased cell debris.

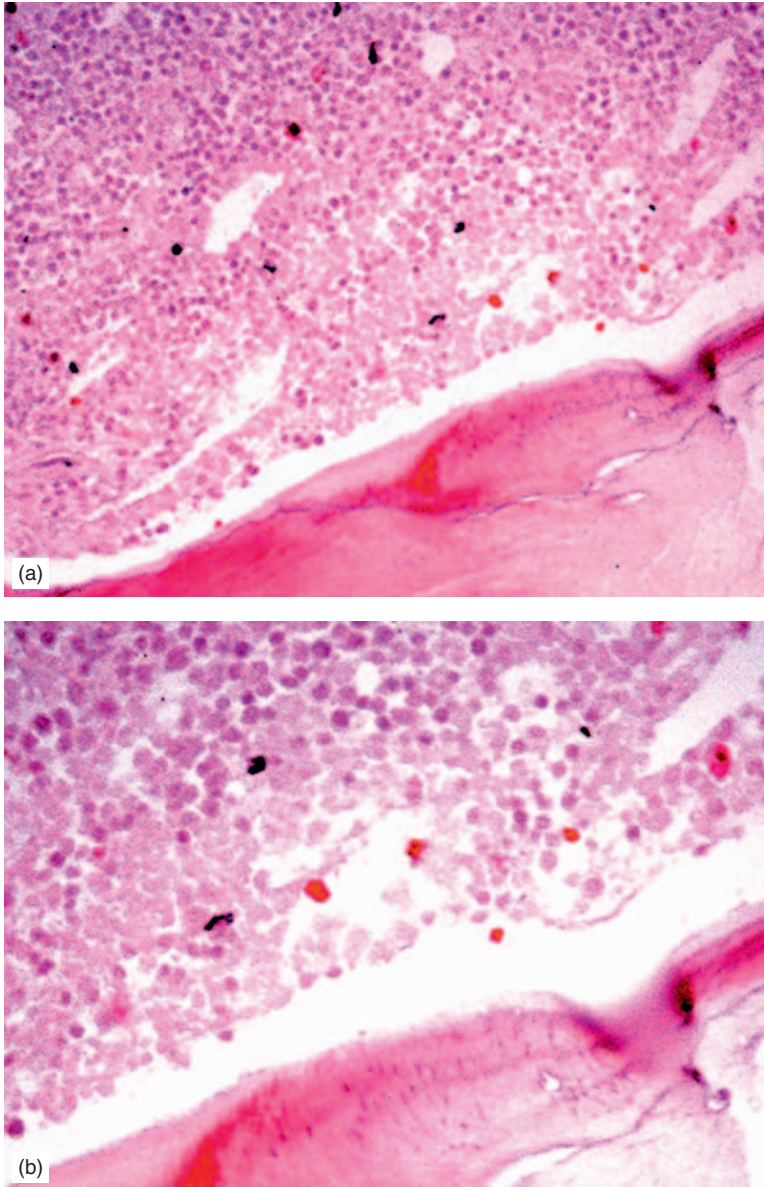


FIGURE 5.2 Low (a) and high (b) power views of a bone marrow biopsy section demonstrating coagulation necrosis. The shadow of the necrotic cells is visible.

frequently observed and is characterized by the preservation of the overall architectural framework of the tissues and the skeleton of the necrotic cells (Figure 5.2). Fibrinoid necrosis consists of the accumulation of an amorphous, granular cell debris and is more often associated with infections. Aspirated necrotic bone marrow contains pyknotic nuclei and blurred outlines of cells in a background of amorphous granular material.

Since hematologic malignancies and metastatic tumors are frequent causes of bone marrow necrosis, the presence of necrosis in bone marrow samples of patients with a history of malignant disease is highly suggestive of bone marrow involvement. In such cases, additional sections and/or samples are recommended.

AMYLOIDOSIS

Amyloid is an extracellular deposit which appears as a hyaline, eosinophilic, amorphous material on H&E sections (Figure 5.3). It predominantly consists of non-branching fibrils composed of polypeptide chains, which on X-ray crystallographic analysis yield a “cross-beta” pleated sheet [20, 21]. Amyloid is often recognized by methyl violet and Congo red histochemical stains. Amyloid deposits appear rose-pink by methyl violet stain and red-orange by Congo red stain. When Congo red stained tissue sections are viewed under a polarizing microscope, amyloid appears as an apple green birefringent deposit (Figure 5.4). It is also

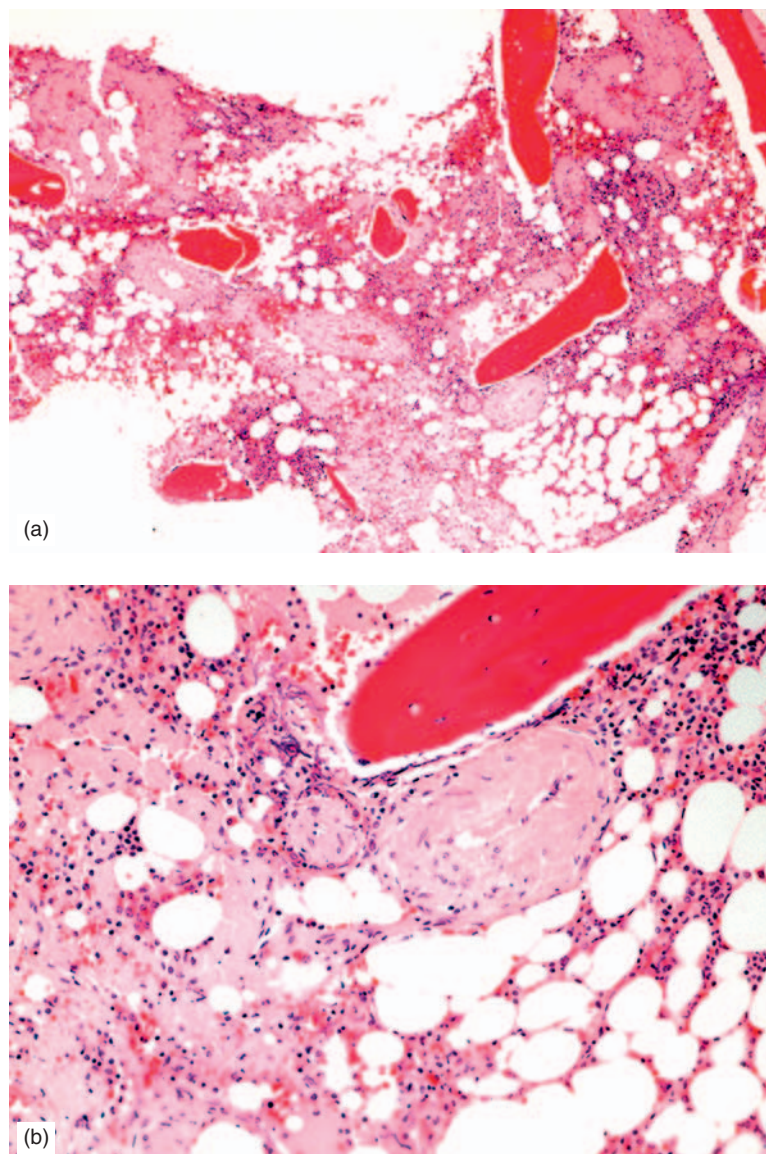


FIGURE 5.3 Low (a) and high (b) power views of a bone marrow biopsy section demonstrating amyloid deposits in the bone marrow space and vessel walls.

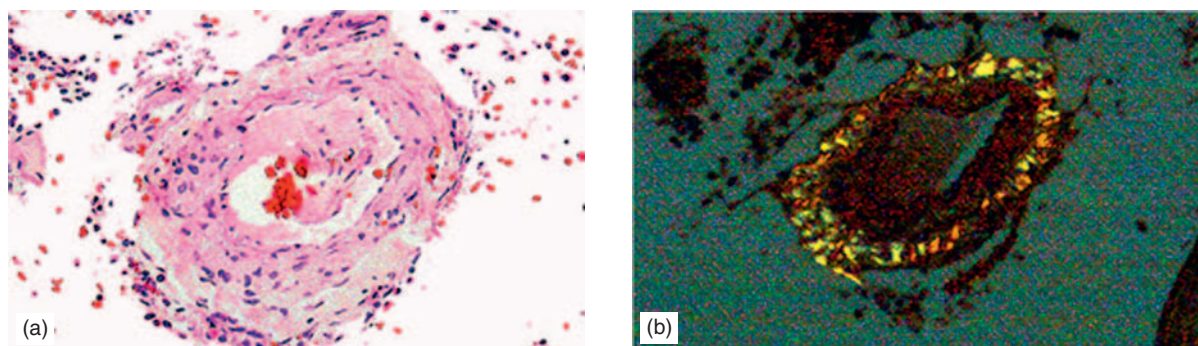


FIGURE 5.4 Amyloidosis. A sclerotic bone marrow blood vessel (a) displays an apple green birefringent deposit by Congo red stain (b).

possible to detect amyloid deposits by using antibodies raised against amyloid components (anti-AA, anti-AL) by immunofluorescence or immunohistochemical techniques.

Amyloid deposits in bone marrow are observed in primary systemic amyloidosis, associated with plasma cell dyscrasias (see Chapter 16), and secondary to chronic inflammatory disorders [22–26]. These deposits are usually multifocal and are found adjacent to or within the walls of small blood vessels. Vascular amyloidosis may lead to ischemia and focal bone marrow hypoplasia. Occasionally, bone marrow involvement with amyloid is extensive.

GRANULOMAS

Granulomas are aggregates of histiocytes which are frequently surrounded by lymphocytes, plasma cells, and eosinophils.

Multinucleated giant cells, which are the result of fusion of closely packed histiocytes, may also be present. Granulomas may show focal areas of necrosis.

The most frequent causes of bone marrow granulomas are mycobacterial and fungal infections and sarcoidosis (Figures 5.5–5.8) [27–30]. However, other infectious and non-infectious conditions, such as viral infections (such as HIV and EBV), syphilis, Q fever, typhoid fever, Legionnaires' disease, Hodgkin and non-Hodgkin lymphomas, autoimmune disorders (Figure 5.9), and drug-induced inflammatory responses may lead to the information of granulomas (Table 5.1) [31–34]. Foreign body granulomas are occasionally seen in bone marrow biopsies which are usually the result of drug abuse or prosthetic pelvic operations (Figure 5.10).

The viral-associated granulomas are usually small, rarely show multinucleated giant cells, and are non-necrotizing [33–35]. Bone marrow granulomas in Q fever

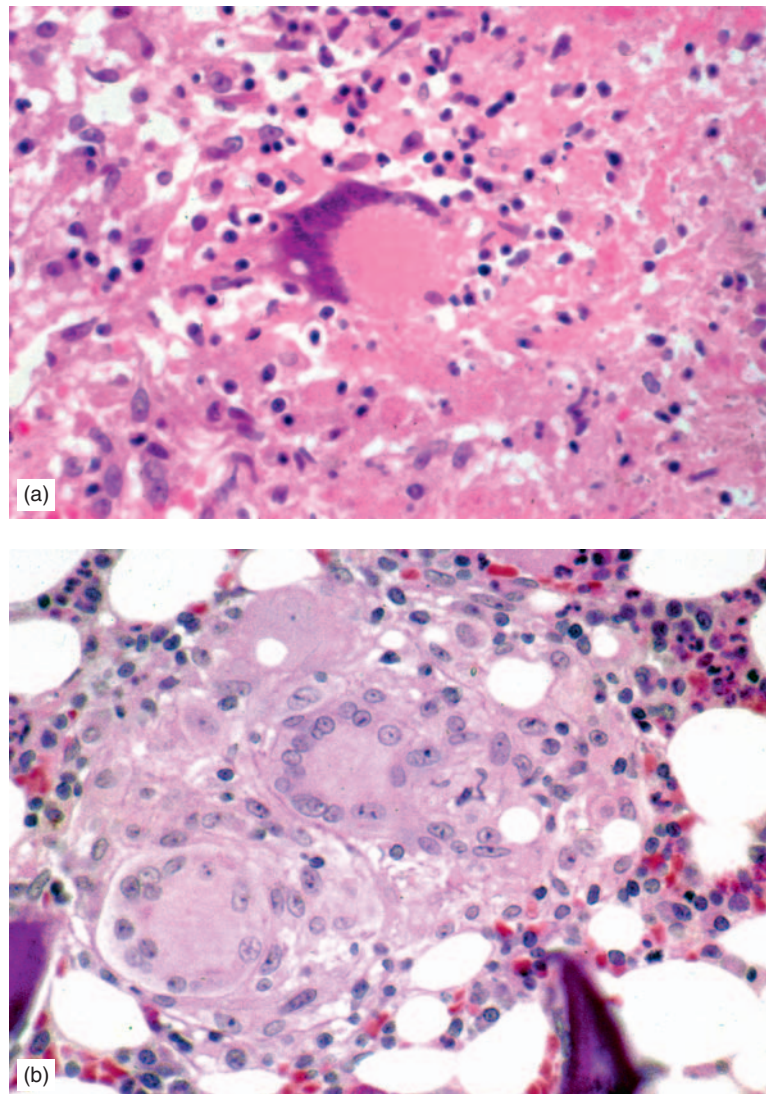


FIGURE 5.5 Bone marrow granulomas. (a) A necrotizing granuloma in a patient with tuberculosis. (b) A non-necrotizing granuloma in a patient with sarcoidosis.

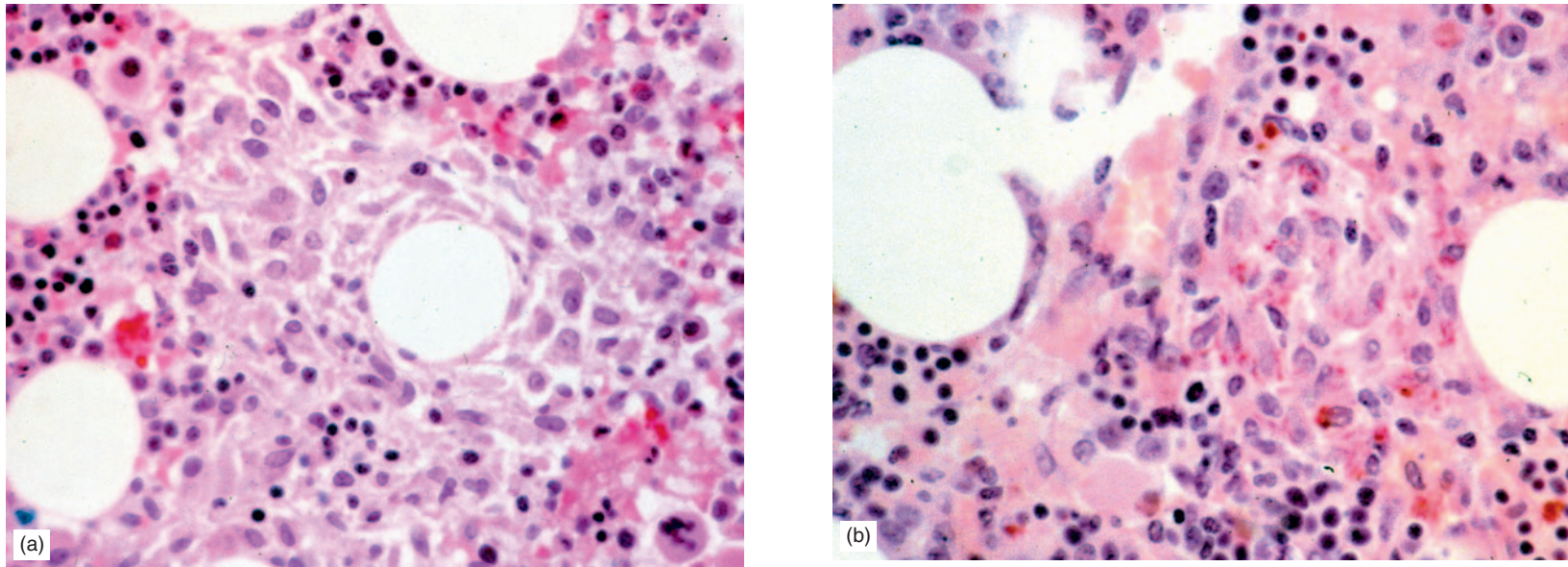


FIGURE 5.6 Bone marrow biopsy section of a patient with AIDS demonstrating a granuloma (a) and numerous bacteria (*Mycobacterium avium-intercellulare*) depicted by acid-fast stain (b).

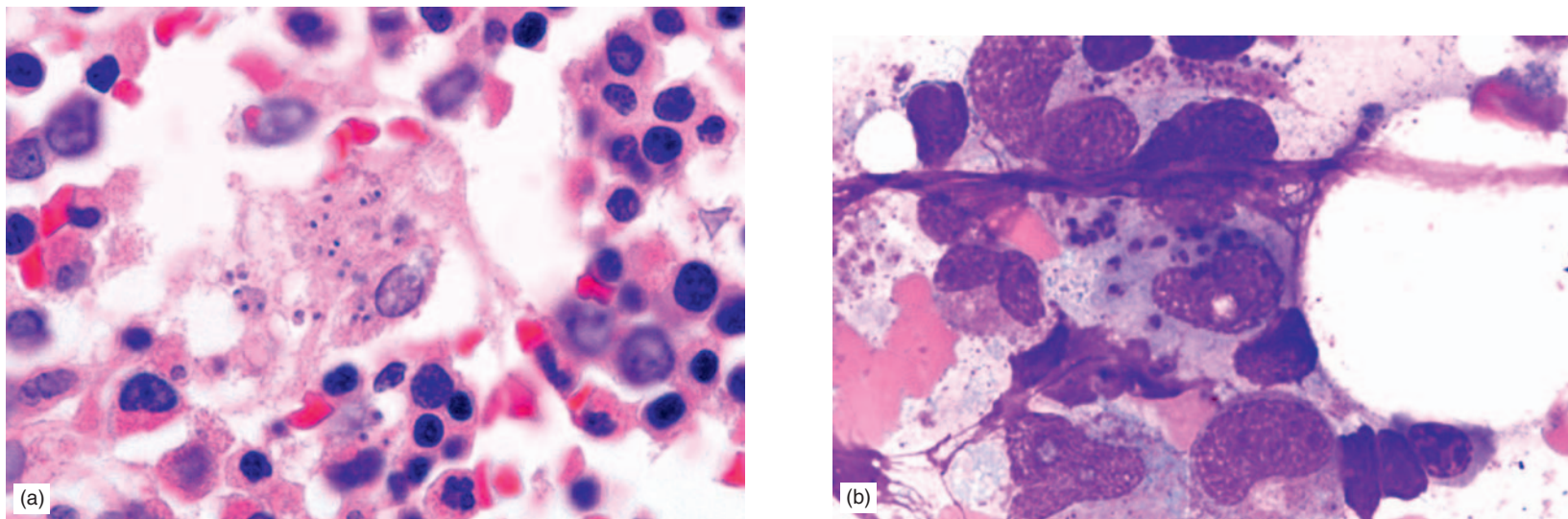


FIGURE 5.7 *Leishmania donovani*. Bone marrow biopsy section (a) and bone marrow smear (b) demonstrating histiocytes containing numerous organisms.

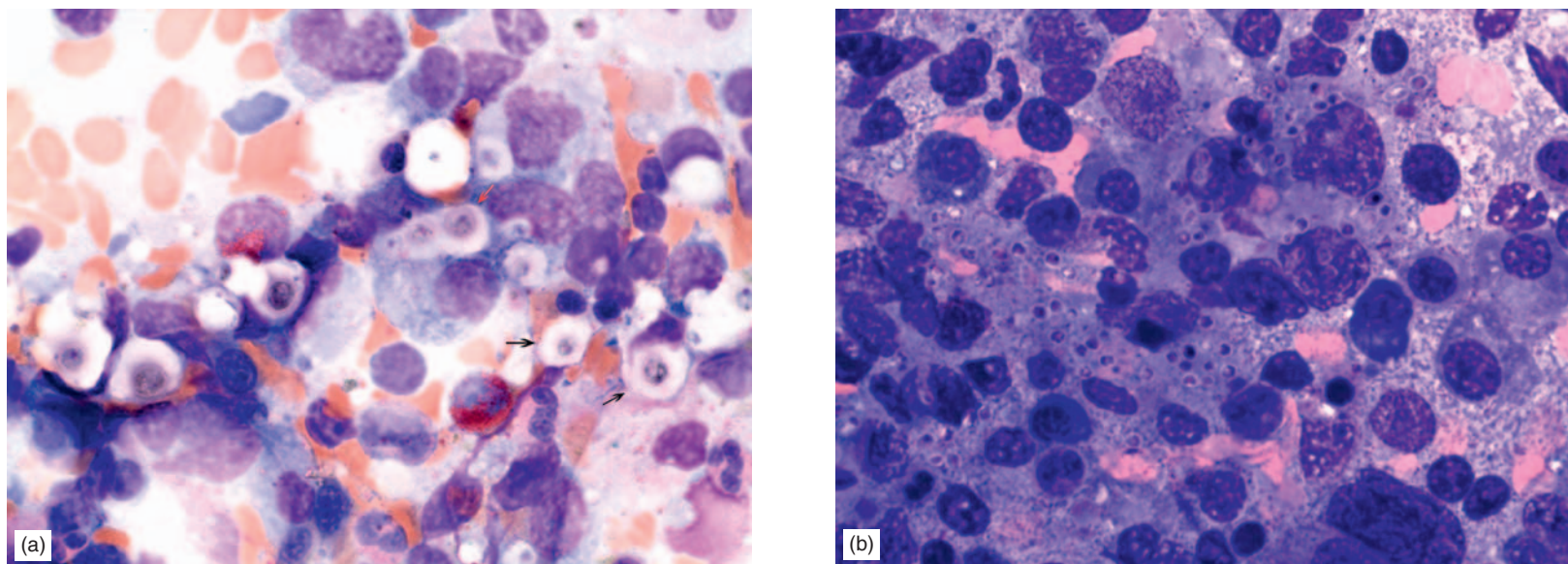


FIGURE 5.8 Bone marrow smears showing involvement with *Cryptococcus neoformans* (a) and *Histoplasma capsulatum* (b).

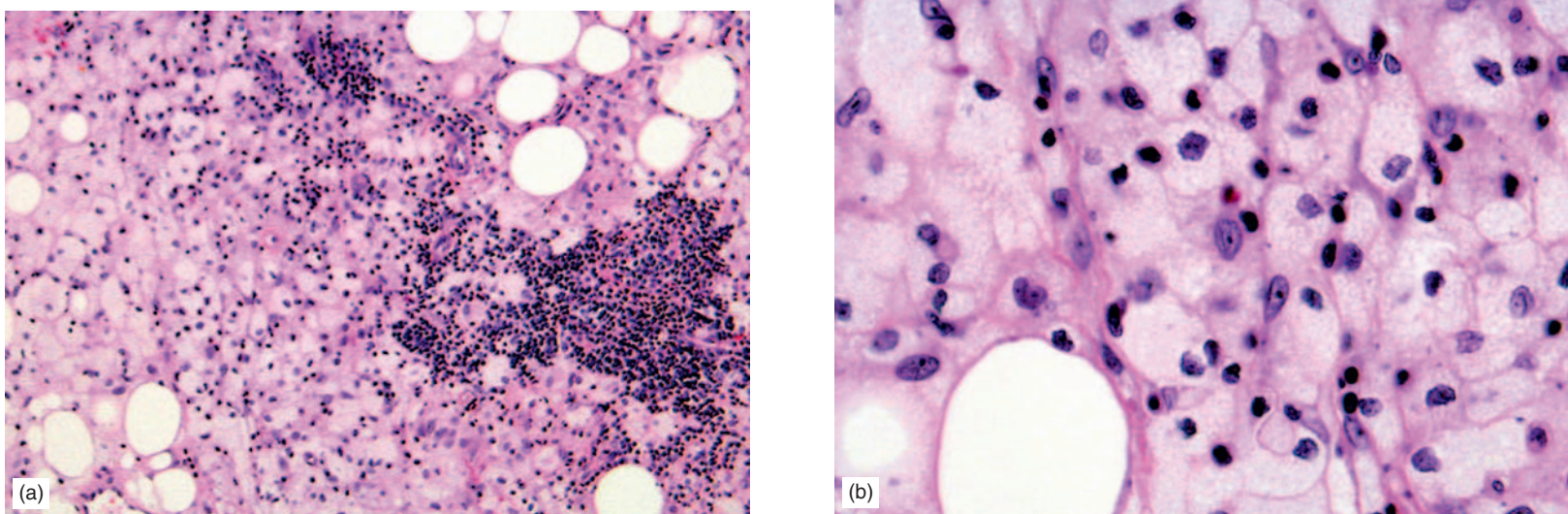


FIGURE 5.9 Low (a) and high (b) power views of a bone marrow biopsy section from a patient with Erdheim–Chester disease demonstrating aggregates of foamy histiocytes.

TABLE 5.1 Conditions associated with bone marrow granulomas.

Infections	Sarcoidosis
Tuberculosis	Hematologic malignancies
<i>Mycobacterium avium-intracellulare</i>	Hodgkin lymphoma
Leprosy	Non-Hodgkin lymphoma
Infectious mononucleosis	Plasma cell myeloma
Cytomegalovirus	
Herpes zoster	Autoimmune disorders
AIDS	
Mycoplasma	Drug-induced
Histoplasmosis	Phenytoin
Cryptococcosis	Procainamide
Brucellosis	Oxyphenbutazone
Typhoid fever	Chlorpropamide
Legionaire's disease	
Q fever	
Rocky Mountain spotted fever	

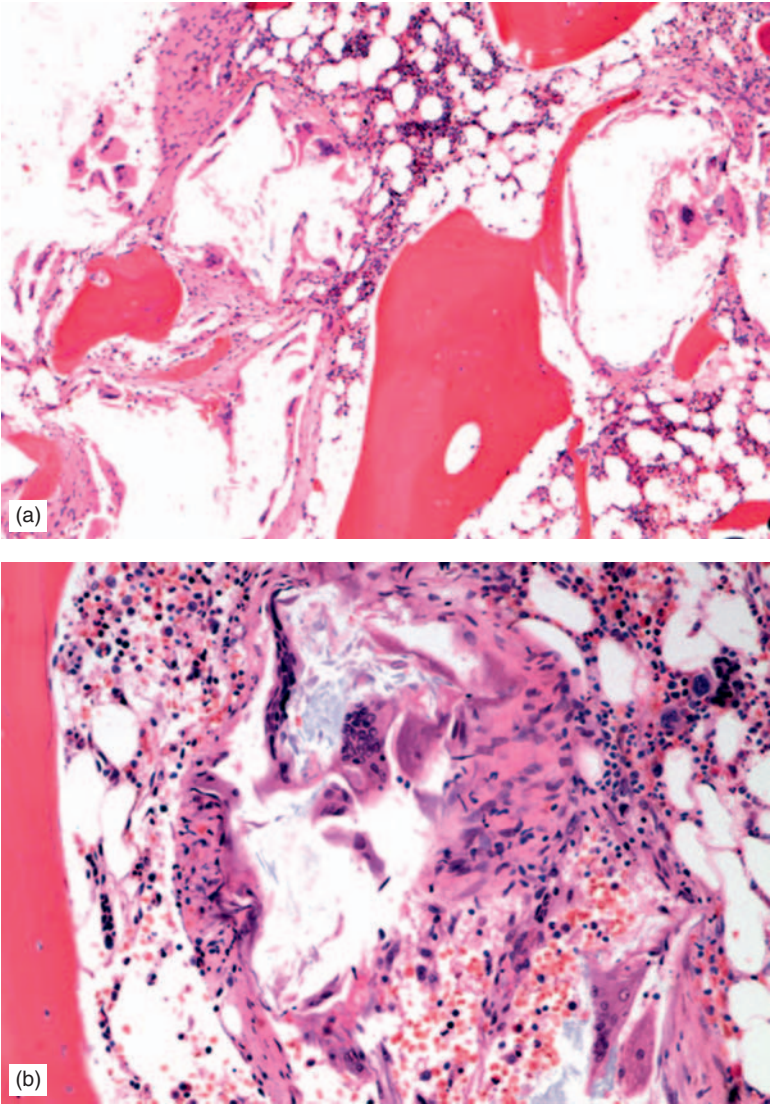


FIGURE 5.10 Low (a) and high (b) power views of a bone marrow biopsy section from a patient with 8-year history of prosthetic pelvic operation showing several cystic structures. These structures contain foreign material surrounded by multinucleated giant cells.

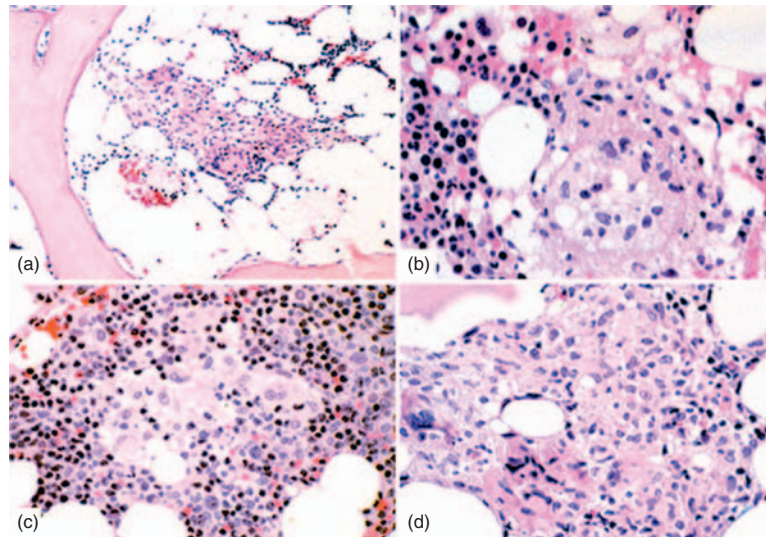


FIGURE 5.11 Small granulomas consisting of epithelioid histiocytes have been observed in the bone marrow of the patients with viral infections (such as HIV and EBV), syphilis, Q fever, typhoid fever, and Legionnaires' disease (a through d).

consist of small clusters of epithelioid histiocytes radially arranged around a fatty tissue containing fibrinoid necrosis (ring granulomas) (Figure 5.11) [36, 37]. The characteristic feature of bone marrow involvement in lepromatous leprosy is the presence of clusters of foamy histiocytes containing lepra bacilli (dirty histiocytes) [38]. Lipogranulomas consist of small aggregates of vacuolated, fat-containing histiocytes in bone marrow sections, frequently observed in association with lymphoid aggregates and plasmacytosis [39].

Granulomas are best detected in bone marrow biopsy sections. It is extremely difficult to detect them in bone marrow smears. A careful search for granulomas is recommended in patients with history of fever of unknown origin, mycobacterial or fungal infections, and in immunocompromised patients. Special stains for mycobacterial and fungal infections are recommended when a granuloma is detected in bone marrow samples.

Certain hematologic disorders, such as peripheral T-cell lymphomas with histiocytic component and metastatic carcinomas composed of cells with abundant cytoplasm (renal cell carcinoma, hepatoblastoma, pheochromocytoma), may resemble granulomas.

BONE MARROW METASTASIS

Bone marrow biopsy sections have a higher detection rate of metastasis than bone marrow clot sections or smears, because metastatic tumor cells trapped in dense fibrosis may not be aspirable. Also, intercellular organization of tumor cells such as glandular structures, rosette formations, and tumor-associated stromal alterations such as fibrosis are usually not detected in bone marrow smears (Figure 5.12). However, it is highly recommended that all various types of bone marrow slides, such as

tissue sections, touch preparations, and smears, are thoroughly examined for the detection of metastatic lesions [1, 40–42].

Metastatic tumor cells tend to clump together, are usually larger than erythroid and myeloid precursors, and may show areas of necrosis (Figures 5.13 and 5.14). Reactive changes such as fibrosis, or the presence of macrophages, lymphocytes, plasma cells, and neutrophils, may be seen within or adjacent to the metastatic site [43, 44].

In bone marrow smears, metastatic tumor cells are usually well defined and in clusters, and are often detected at the periphery of the marrow particles. Small round cell tumors (oat cell carcinoma, neuroblastoma, retinoblastoma, rhabdomyosarcoma, Ewing's sarcoma) may resemble hematopoietic blast cells. However, they are usually larger than the hematopoietic blasts, often show significant nuclear pleomorphism, and may also show characteristic morphologic structures, such as organoid pattern (oat cell carcinoma) or rosette formation (neuroblastoma) (Figure 5.14c). In addition, small cell tumors may demonstrate specific features by electron microscopy, immunophenotypic studies, molecular and cytogenetic studies, or *in vitro* culture [45–50]. For example, the diagnosis of a metastatic neuroblastoma is facilitated by the existence of secretory granules by electron microscopy, the presence of cellular catecholamines and neurofilaments by immunohistochemistry, and by demonstrating the outgrowth of neurites in tissue culture.

The presence of bone marrow fibrosis in patients with a history of malignancy is strongly suspicious for metastasis. Metastatic carcinomas, particularly of breast origin, are frequently associated with bone marrow fibrosis. Clusters and strands of tumor cells are often trapped in a dense, highly collagenized, fibrous tissue. Sometimes, fibrosis may make the detection of small metastatic foci difficult. In such cases, examination of additional tissue sections may be helpful. As mentioned earlier, metastatic carcinomas composed of cells with abundant cytoplasm may resemble granulomas.

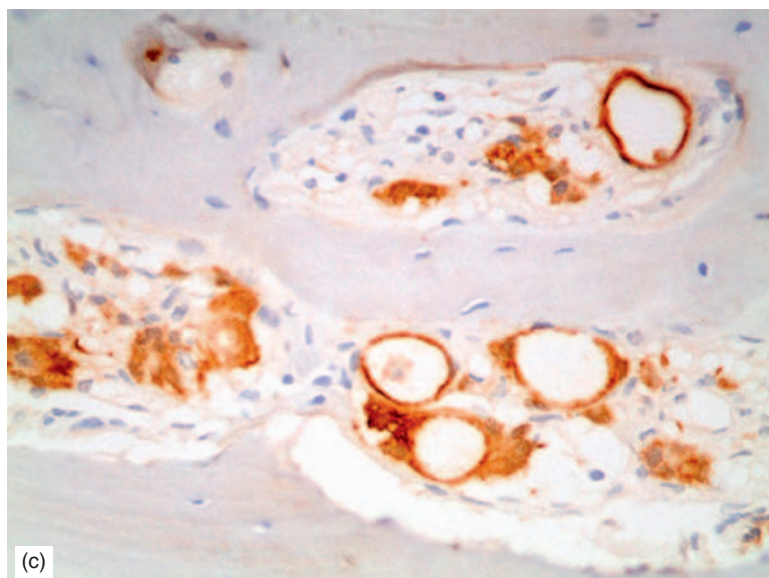
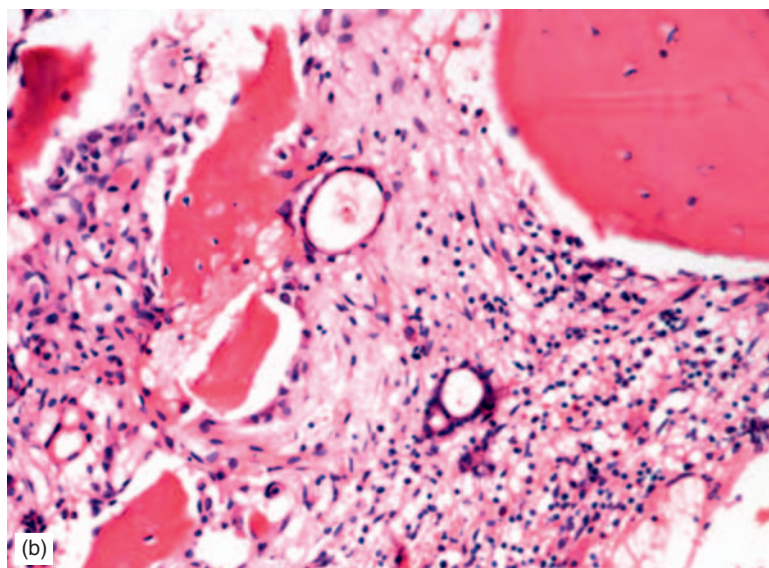
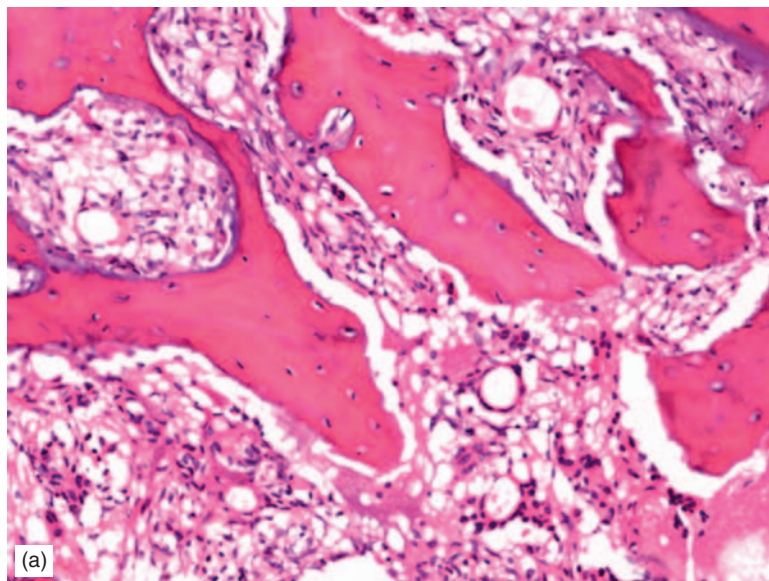


FIGURE 5.12 Bone marrow biopsy section, (a) low power and (b) high power, demonstrating sclerosis of the bone trabeculae and extensive fibrosis with scattered metastatic glandular structures expressing cytokeratin (c).

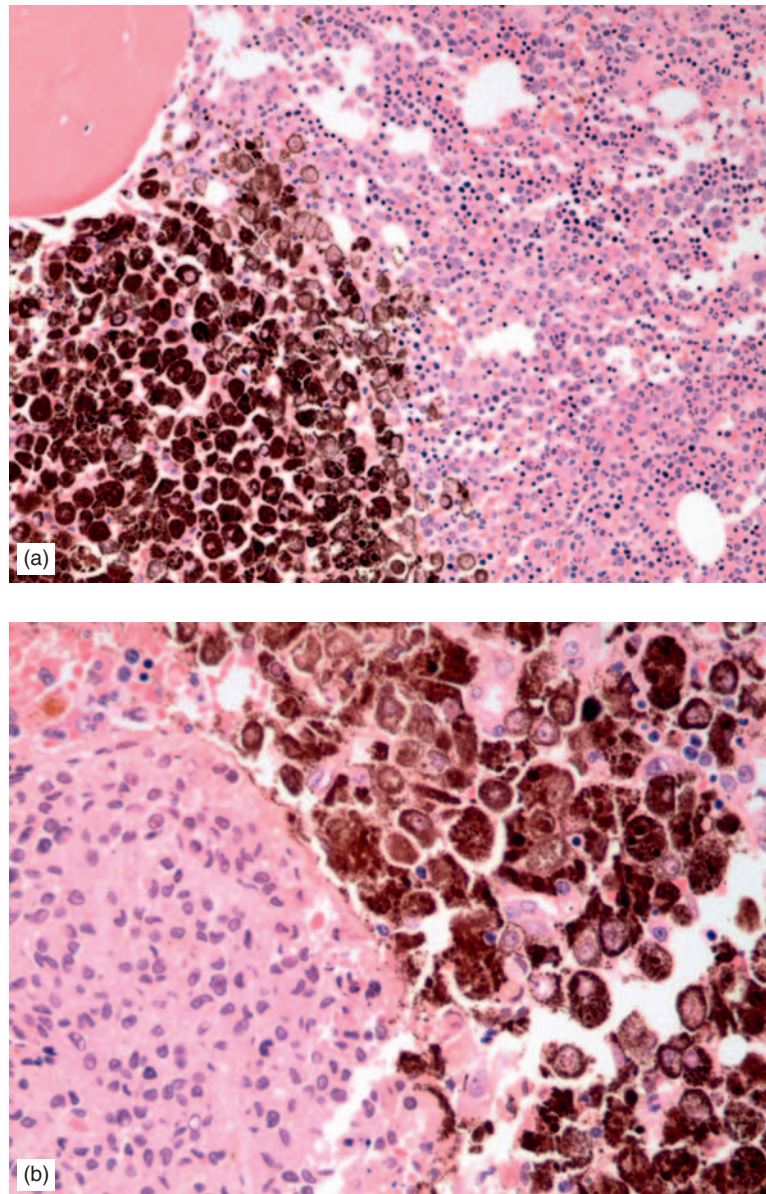


FIGURE 5.13 Bone marrow biopsy section depicting metastatic melanoma (a). The melanoma consists of pigmented and non-pigmented components (b).

Immunophenotypic studies are useful in (1) distinguishing metastatic neoplasms from primary bone marrow malignancies and reactive proliferations, (2) classification of metastatic tumors and their possible primary sites, and (3) detection of small, occult metastatic lesions [45, 47]. For example, tumors of epithelial origin express cytokeratin and are negative for CD45; metastatic prostate carcinoma is positive for prostate specific antigen (PSA) and prostatic acid phosphatase, and metastatic rhabdomyosarcoma may demonstrate myosin, desmin, and/or myoglobin.

POST-THERAPEUTIC CHANGES

The morphologic changes of the bone marrow after chemotherapy and/or irradiation are the result of rapidly progressive cellular death and a transient ineffective hematopoiesis. These changes include marked hypocellularity, fibrinoid necrosis, edema, dilated sinuses, multilobulated adipocytes, new bone formation, mild to moderate increase in reticulin fibers, and increased number of macrophages, frequently with phagocytic particles (Figures 5.15 and 5.16) [1, 51].

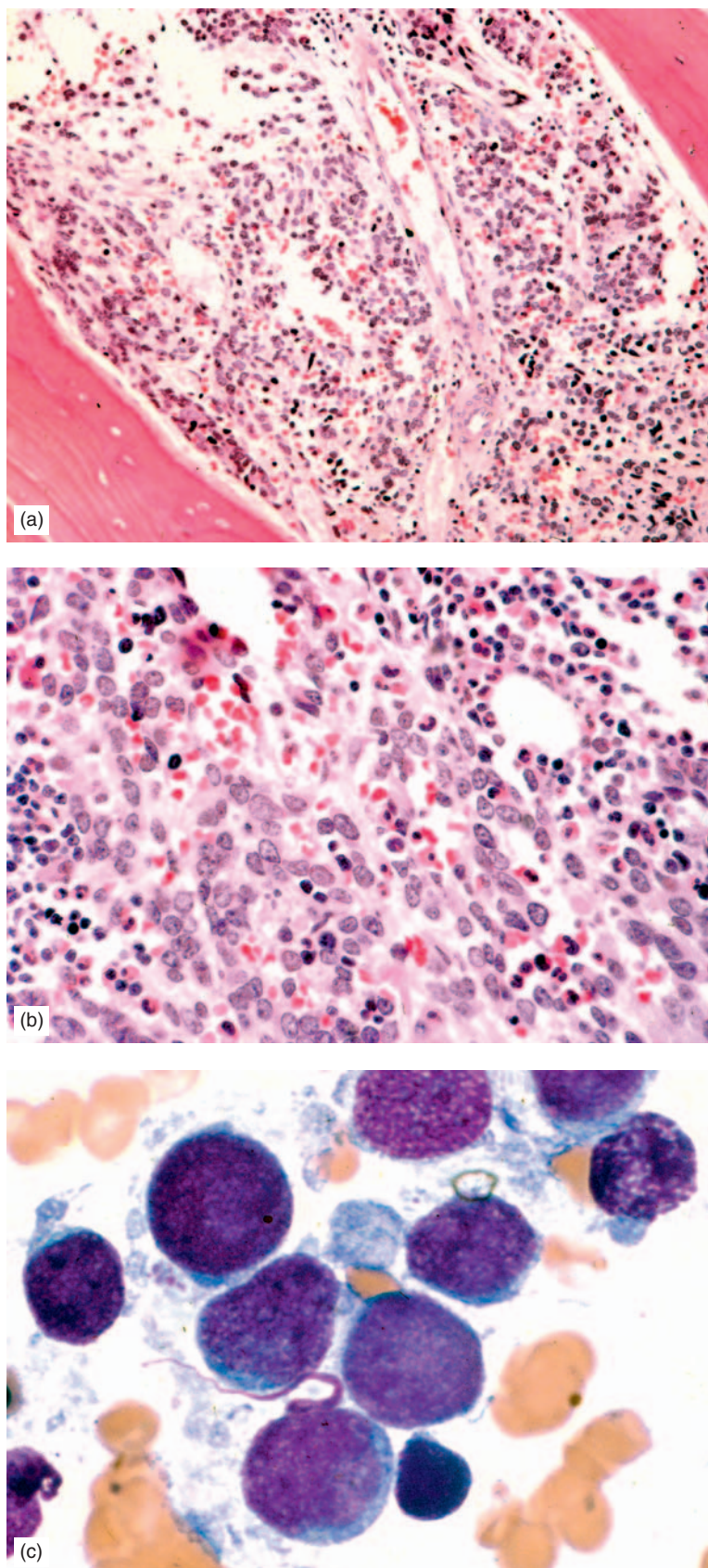


FIGURE 5.14 Metastatic neuroblastoma demonstrating clusters of small round tumor cells. Bone marrow biopsy section: (a) low power and (b) high power views. (c) Bone marrow smear.

Morphologic evidence of post-therapy bone marrow regeneration usually appears 1–2 weeks after therapy. Usually, erythroid and myeloid precursors appear sooner than megakaryocytes. Myeloid precursors are usually adjacent to bone, whereas erythroid clusters are far from bone trabeculae and are surrounded by fatty tissue. Rapid bone marrow regeneration is often associated with left-shifted hematopoiesis and increased hematogones [1].

Bone marrow changes following growth factor and/or interleukin therapy usually consist of increased cellularity, left shift, and may be associated with some degree of dysplastic changes. IL-3 therapy may occasionally cause marrow fibrosis, and GM-CSF and G-CSF cause myeloid preponderance and left shift, sometimes with marked eosinophilia [52–55].

BONE AND STROMAL CHANGES

Bone marrow stroma and bone play an important role in support and regulation of hematopoiesis [56–60]. However, pathological changes of hematopoiesis may significantly affect structure and function of the bone marrow stroma and the surrounding bone trabeculae.

Bone Changes

Hematopathologists and hematologists may overlook the bone changes when they review bone marrow biopsy sections. These changes are usually of two types: (1) associated with decreased bone formation (osteopenia) and (2) associated with increased bone formation (osteosclerosis).

Conditions Associated with Osteopenia

The receptor activator of nuclear factor- κ B ligand (RANKL) is a pivotal regulator of osteoclast activity, and its inhibition may lead to osteopenia and hypercalcemia [61–65]. Osteopenia (osteoporosis, osteolysis) is caused by various conditions including circulatory disturbances, metabolic deficiencies, endocrine imbalance or deficiency, dialysis, inflammatory conditions, inactivity and immobilization, and the expansion of bone marrow space caused by metastasis, plasma cell myeloma, leukemia/lymphoma, and extensive bone marrow hypercellularity due to erythroid hyperplasia [1, 66]. Alteration of RANKL activity has been reported in many of these conditions, particularly plasma cell myeloma [67–69].

In osteoporosis, the bone marrow space is expanded at the expense of bone trabeculae. The bone trabeculae are thin and far apart. In osteomalacia the osteoid is partially decalcified.

Conditions Associated with Osteosclerosis

Osteosclerosis is demonstrated in bone marrow biopsy sections by increased thickness of the bone trabeculae. It is observed in chronic idiopathic myelofibrosis, leukemias, metastatic cancers, Erdheim–Chester disease, and mastocytosis (Figure 5.17) [1, 70, 71]. Osteopetrosis, an inherited disease which occurs in both autosomal dominant and recessive forms, is also characterized by increased bone density [72, 73].

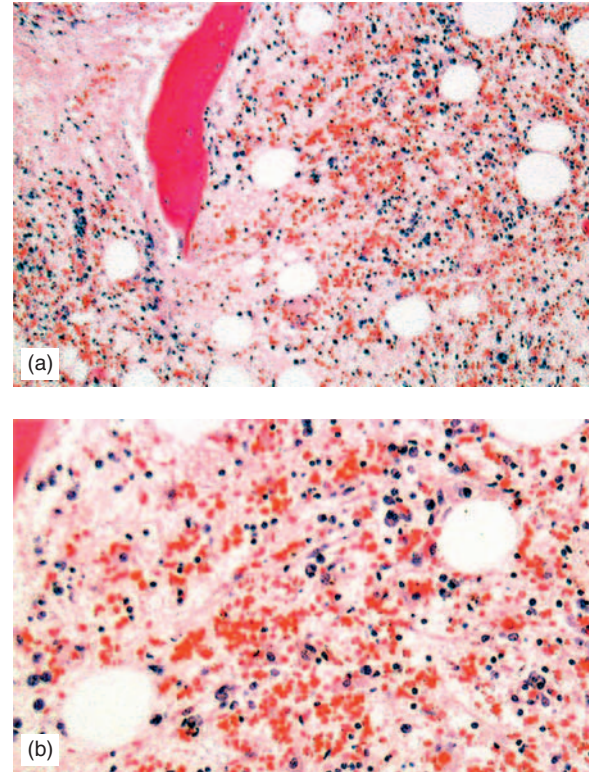


FIGURE 5.15 Post-chemotherapy bone marrow sections are hypocellular with areas of necrosis, scattered hematopoietic precursors, stromal tissue, and minimal fat: (a) low power and (b) high power.

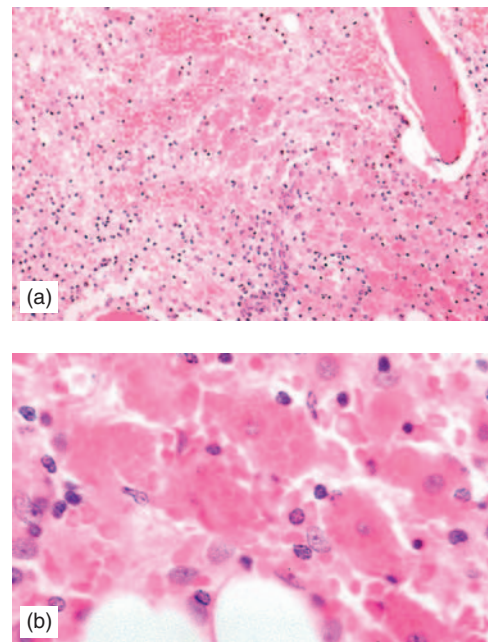


FIGURE 5.16 Bone marrow biopsy sections after chemotherapy or irradiation are hypocellular and may demonstrate necrosis, edema, and increased number of histiocytes (a). Higher power view (b) shows some hemophagocytic histiocytes.

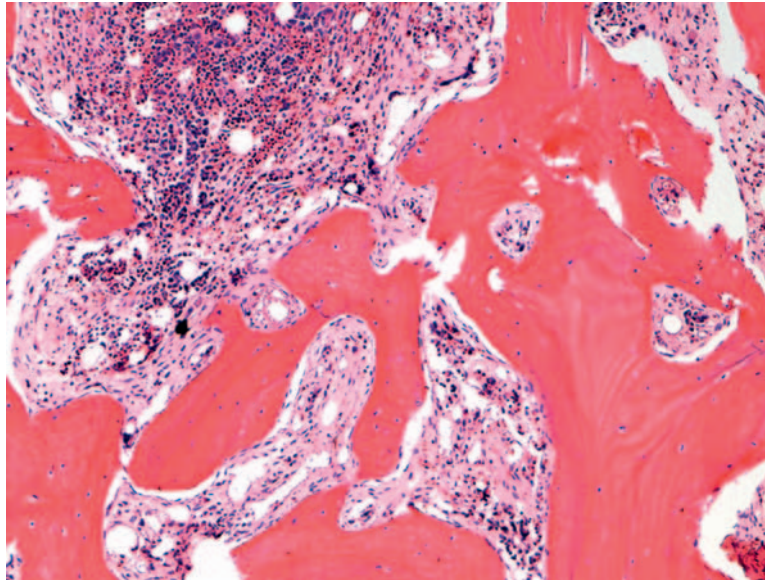


FIGURE 5.17 Bone marrow biopsy section from a patient with mastocytosis showing sclerotic bone trabeculae and areas of fibrosis.

Previous Biopsy Site (Repair)

The recovery to the damaged bone marrow following biopsy is relatively fast and complete. However, occasionally, when several biopsy attempts are made at short intervals, the biopsy may show features of tissue repair, such as neovascularization and proliferation of the fibroblasts (granulation tissue), edema, increased number of macrophages, and evidence of new bone formation (Figure 5.18). In addition, fragments of necrotic bone or bone marrow tissue may be still present. The reparative changes may mimic myelofibrosis, granuloma, or Paget's disease.

Bone Marrow Fibrosis

Bone marrow fibrosis is a common phenomenon and is observed in various pathological conditions such as myeloproliferative and myelodysplastic disorders, leukemias, lymphomas, mastocytosis, paroxysmal nocturnal hemoglobinuria, plasma cell myeloma, Gaucher's disease, granulomas, metastatic tumors, hyperparathyroidism, chronic renal failure,

osteopetrosis, autoimmune disorders, and Paget's disease (Table 5.2) (Figures 5.19 and 5.20). Fibrosis may be focal (e.g. fibrosis associated with chronic renal failure or mastocytosis) or diffuse (e.g. fibrosis associated with myeloproliferative disorders).

Bone marrow fibrosis is a non-clonal reactive process and is caused by the release of fibroblastic growth factors by megakaryocytes, platelets, histiocytes, and other cells.

Vascular Changes

Vascular inflammatory changes such as arteritis, arteriolitis, and granulomatous vasculitis may involve bone marrow as part of a systemic process (Figure 5.21). Similarly, atherosclerotic and thrombotic lesions may be detected in bone marrow biopsy. Tumor emboli are the major sources of bone marrow metastasis, and when they are extensive may cause microangiopathic hemolytic anemia. As mentioned earlier, bone marrow amyloidosis may involve vascular structures.

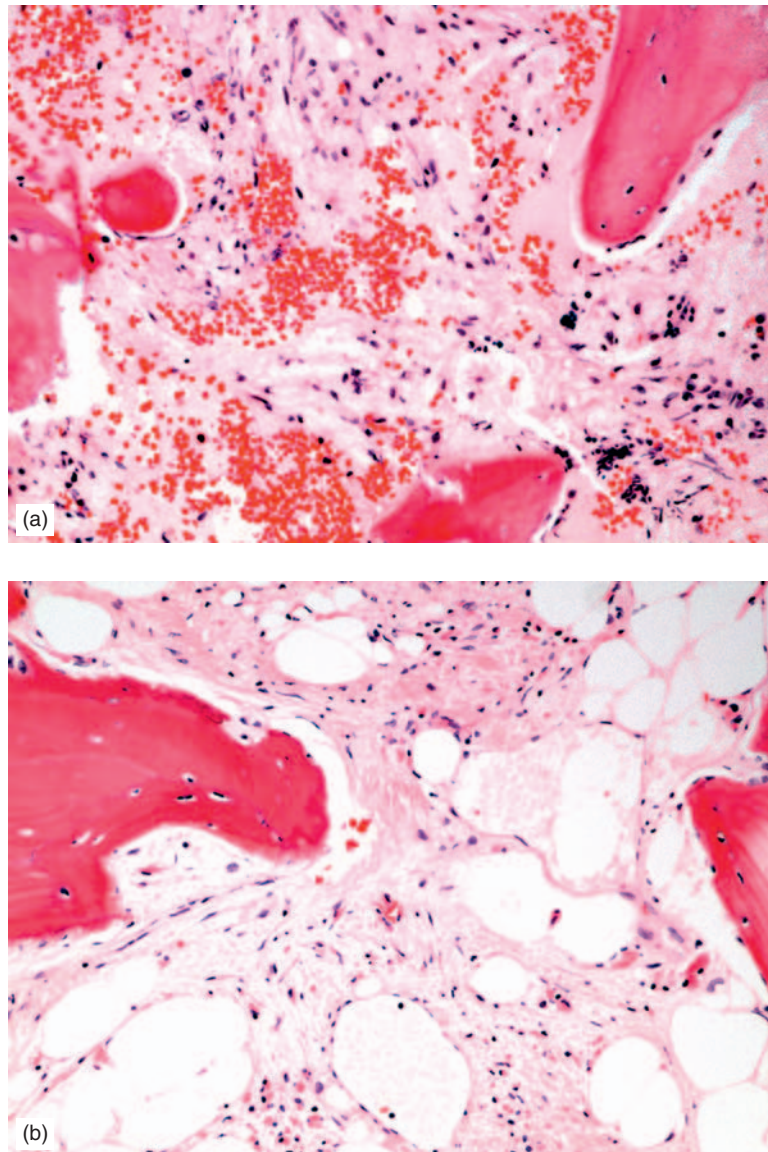


FIGURE 5.18 Bone marrow biopsy sections from previous biopsy sites may show evidence of bone remodeling, granulation tissue formation (a) and/or fibrosis (b).

TABLE 5.2 Conditions associated with bone marrow fibrosis.

Primary hematologic disorders
Chronic myeloproliferative disorders
Myelodysplastic syndromes
Paroxysmal nocturnal hemoglobinuria
Aplastic anemia
Leukemias
Lymphomas
Plasma cell myeloma
Mastocytosis
Gray platelet syndrome
Metastatic lesions
Lysosomal storage diseases
Inflammatory and repair processes
Granulomas
Osteomyelitis
Autoimmune disorders
Following bone marrow necrosis
Following bone marrow radiation
Previous bone marrow biopsy site
Metabolic disorders
Osteomalacia
Osteopetrosis
Chronic renal failure
Primary hyperparathyroidism
Paget's disease

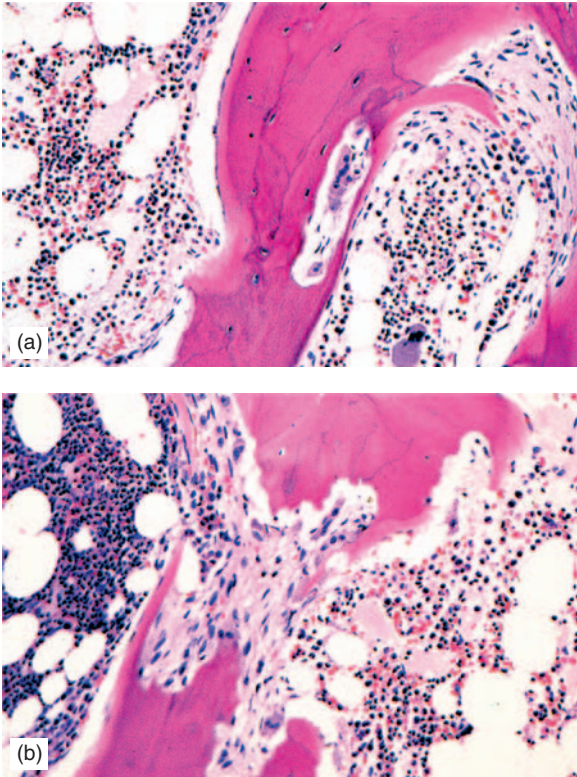


FIGURE 5.19 Bone marrow biopsy section from a patient with chronic renal failure demonstrating paratrabecular fibrosis, bone resorption and remodeling, and the presence of osteoclasts.

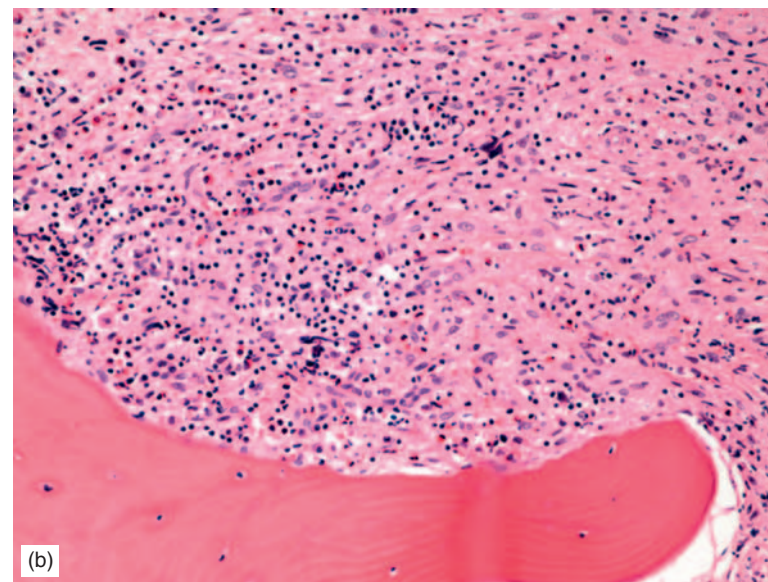
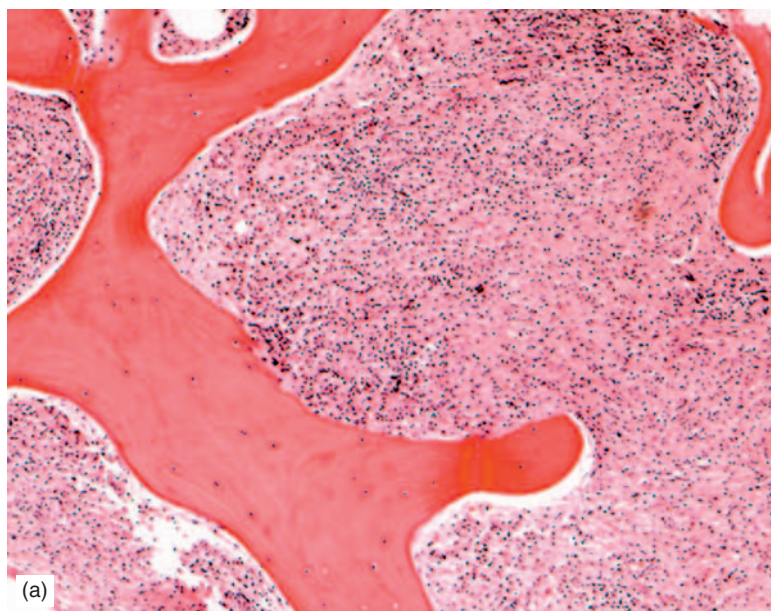


FIGURE 5.20 Occasionally, autoimmune disorders may be associated with extensive bone marrow fibrosis: (a) low power and (b) high power.

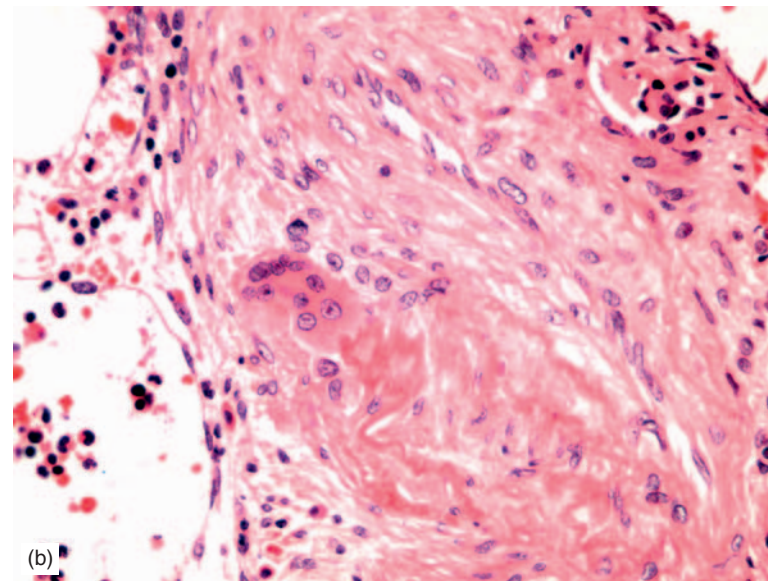
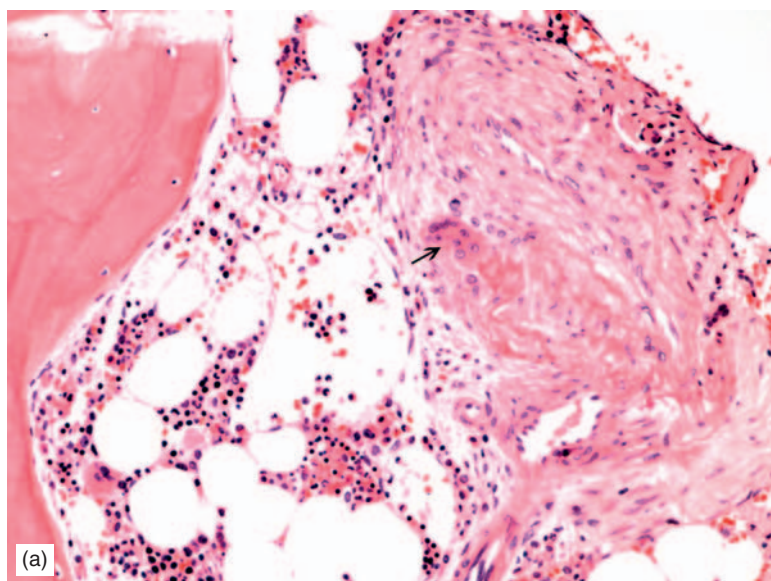


FIGURE 5.21 A vascular structure in a bone marrow biopsy section showing features of giant cell arteritis with a large multinucleated giant cell in the sclerotic wall: (a) low power and (b) high power.

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Reactive Lymphadenopathies

Faramarz Naeim

The reactive lymphadenopathies represent hyperplasia of different lymph node components and therefore demonstrate particular structural patterns such as follicular hyperplasia, paracortical hyperplasia, sinus histiocytosis, granulomas, or a mixed pattern (Table 6.1) [1–3].

FOLLICULAR HYPERPLASIA

In follicular hyperplasia, the lymph node enlargement is primarily due to the increased number and size of the follicles [1, 2]. Germinal centers

TABLE 6.1 Patterns of reactive lymphadenitis.

Patterns	Examples
<i>Follicular hyperplasia</i> Enlargement of germinal centers, follicles in variable size and shapes, intact mantle zones	Rheumatoid arthritis Castleman's disease Early HIV infection Bacterial infections
<i>Paracortical hyperplasia</i> Expansion of interfollicular areas, increased high endothelial venules, mostly T-cells	Infectious mononucleosis Postvaccinal lymphadenitis Drug-induced
<i>Sinus pattern</i> Expansion of sinusoidal spaces with proliferation of histiocytes or monocytoid B-lymphocytes	Sinus histiocytosis Rosai–Dorfman disease Whipple's disease Monocytoid B-cell hyperplasia
<i>Granulomatous lymphadenitis</i> Granulomas may be of epithelioid type, may show caseous necrosis, or may be suppurative	Tuberculosis Sarcoidosis Cat-scratch fever Fungal infections
<i>Mixed pattern</i>	Dermatopathic lymphadenitis Toxoplasmosis Kikuchi's disease Kimura's disease

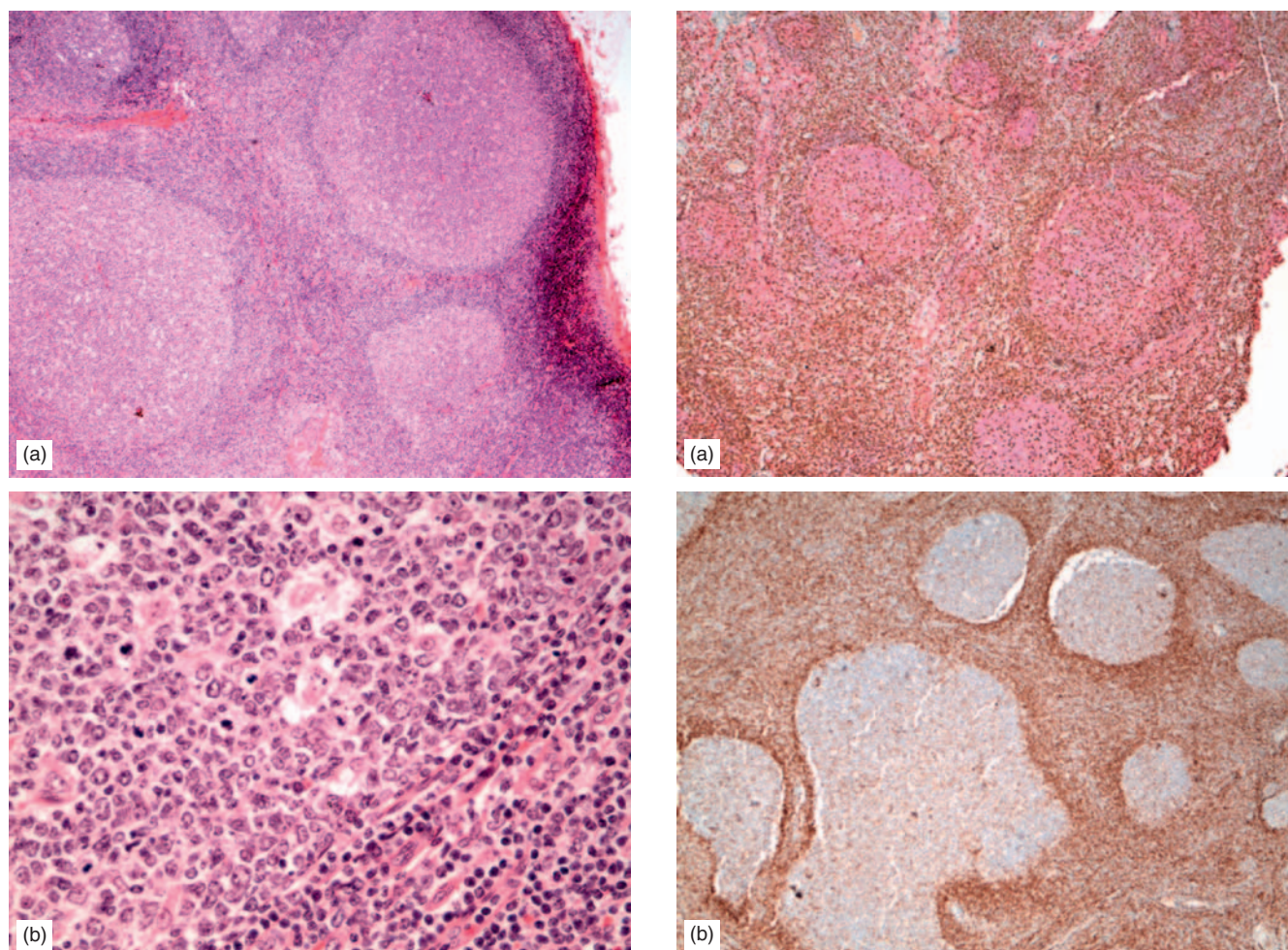


FIGURE 6.1 (a) Follicular hyperplasia with the expansion of germinal centers. (b) High power view of a germinal center showing centrocytes, centroblasts, tingible body macrophages, and mitotic figures.

are expanded and follicles appear in different sizes and shapes and are surrounded by well-defined mantle zones. Follicles occupy cortical, paracortical, and even sometimes medullary zones, but they are usually separated from one another by interfollicular lymphoid tissues. The expanded germinal centers show numerous tingible body macrophages intermixed with centrocytes and centroblasts and are characterized by the CD21-positive follicular dendritic cell network, and lack of the expression of BCL-2 (Figures 6.1 and 6.2) [1, 2, 4]. Mitotic figures and apoptotic bodies are frequent. Plasma cells may be present within the germinal centers, occasionally in large numbers. Interfollicular areas often show vascular proliferation and a mixture of small lymphocytes, plasma cells, and immunoblasts, sometimes with increased number of histiocytes and eosinophils. Medullary plasmacytosis is a frequent finding. As discussed in Chapter 14, differential diagnosis of follicular hyperplasia from follicular lymphoma is sometimes difficult [5, 6]. The major distinguishing features between these two categories are summarized in Table 6.2. Castleman's disease, rheumatoid arthritis (RA), and various infections, such as syphilis, are often associated with follicular hyperplasia. The morphologic features of RA and Castleman's disease are briefly discussed later.

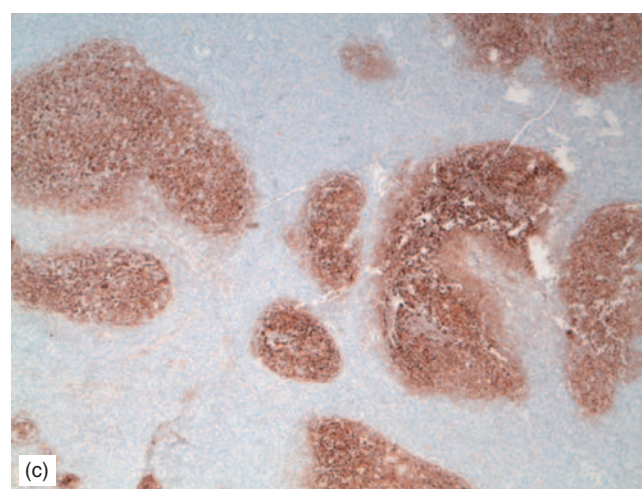


FIGURE 6.2 Immunohistochemical studies of hyperplastic follicles: (a) B-cells (CD20-positive, red) are the predominant cells in the germinal centers and T-cells (CD3-positive, brown) are the predominant cells in the interfollicular areas. (b) Reactive follicles do not express BCL-2 and (c) CD21 expression represents the network of follicular dendritic cells.

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is an autoimmune, chronic systemic disorder affecting women more than men (male:female

TABLE 6.2 Morphologic features distinguishing follicular lymphoma (FL) from reactive follicular hyperplasia (RFH).

Features	FL	RFH
<i>Follicles</i>	High density per unit area	Low density per unit area
	Back to back or merging, often with loss of mantle zone	Separated, with preservation of mantle zone
	Lack of polarity	Presence of polarity
	Monoclonal	Polyclonal
	Commonly BCL-2 positive	BCL-2 negative
	Low Ki-67 fraction	High Ki-67 fraction
<i>Interfollicular areas</i>	Presence of CD10 positive cells	Absence of CD10 positive cells
	Presence of BCL-6 positive cells	Absence of BCL-6 positive cells

ratio of about 1:3) [7, 8]. Most patients are over 30 years of age. The juvenile form of RA is often associated with high spiking fevers and transient rashes (Still's disease) [9, 10]. The lymph node biopsy sections show prominent follicular hyperplasia with sinus histiocytosis (Figure 6.3) [2, 11, 12]. Increased plasma cells are frequently observed in the interfollicular regions. The lymphadenopathy in longstanding cases may be associated with the deposition of periodic acid Schiff (PAS)-positive hyaline material (negative for Congo red stain) with partial or complete replacement of the lymphoid tissue [2].

Castleman's Disease

Castleman's disease, also known as angiofollicular hyperplasia or giant lymph node hyperplasia [1, 2, 13], consists of two forms: (1) localized type and (2) multicentric type. The localized type of Castleman's disease is much more frequent than the multicentric type, usually involving mediastinum. Other sites such as axillary and cervical lymph nodes, skeletal muscles, and pulmonary parenchyma have been involved less frequently [1, 2]. There are two morphologic variants of Castleman's disease: (1) hyaline vascular type and (2) plasma cell type.

Hyaline Vascular Type

The hyaline vascular type of Castleman's disease is the common variant accounting for over 90% of cases in one large study [13–17]. The lesion is characterized by abnormal germinal centers, expanded mantle zones, and increased interfollicular vascularity. Germinal centers contain hyalinized vascular structure and show increased number of follicular dendritic cells (Figure 6.4). Follicular dendritic cells may appear large and bizarre. The mantle zone area is often expanded and concentrically arranged, creating an “onion skin” pattern (Figure 6.4b). The expanded mantle zones may sometimes completely obscure the germinal center [1]. Blood vessels are prominent in the interfollicular areas, some with prominent endothelial cells (Figure 6.5). Small lymphocytes and scattered plasma cells are found in between vascular structures.

The hyaline vascular type, because of the expansion of mantle zone areas, may resemble mantle cell lymphoma. Mantle cell lymphoma is monoclonal and demonstrates Ig light chain restriction, expresses BCL-1, and is usually devoid of hyalinized vessels.

Plasma Cell Type

The plasma cell type of Castleman's disease is less frequent (~10%) and is often associated with polyclonal gamma globulinemia, increased serum levels of IL-6, and elevated erythrocyte sedimentation rate. Anemia and elevated erythrocyte sedimentation rate are frequent findings. The most frequent site of involvement is the abdomen, particularly in the small bowel mesentery [1, 2, 18]. Lymph node sections show follicular hyperplasia with a well-defined mantle zone, surrounded by sheets of mature plasma cells and scattered immunoblasts. Vascular proliferation or hyalinization is usually absent. In approximately 40% of the cases the plasma cells are monotypic and express Ig lambda light chain [1, 19]. The plasma cell type resembles other follicular hyperplasias, such as those associated with RA, or other autoimmune disorders. Diagnosis of Castleman's disease of plasma cell type is a diagnosis of exclusion, when all other possible causes of follicular hyperplasia have been ruled out [1, 2].

PARACORTICAL (INTERFOLLICULAR) PATTERN

Lymphadenitis with paracortical pattern is characterized by the expansion of the interfollicular areas with a mixture of small lymphocytes, immunoblasts, and increased high endothelial venules. T-cells are the predominant cell type, but there are variable numbers of B-immunoblasts and plasma cells. Infectious mononucleosis, postvaccinal lymphadenitis, and drug-induced hypersensitivity reactions are classical examples of lymphadenitis with paracortical pattern.

SINUS PATTERN

Sinus Histiocytosis

Sinus histiocytosis is a relatively common phenomenon associated with a garden variety of infectious and inflammatory conditions. It is characterized by the expansion of sinuses due to the increased number of histiocytes (Figure 6.6). Pulmonary hilar lymph nodes in heavy smokers and elderly individuals contain a large number of sinus histiocytes

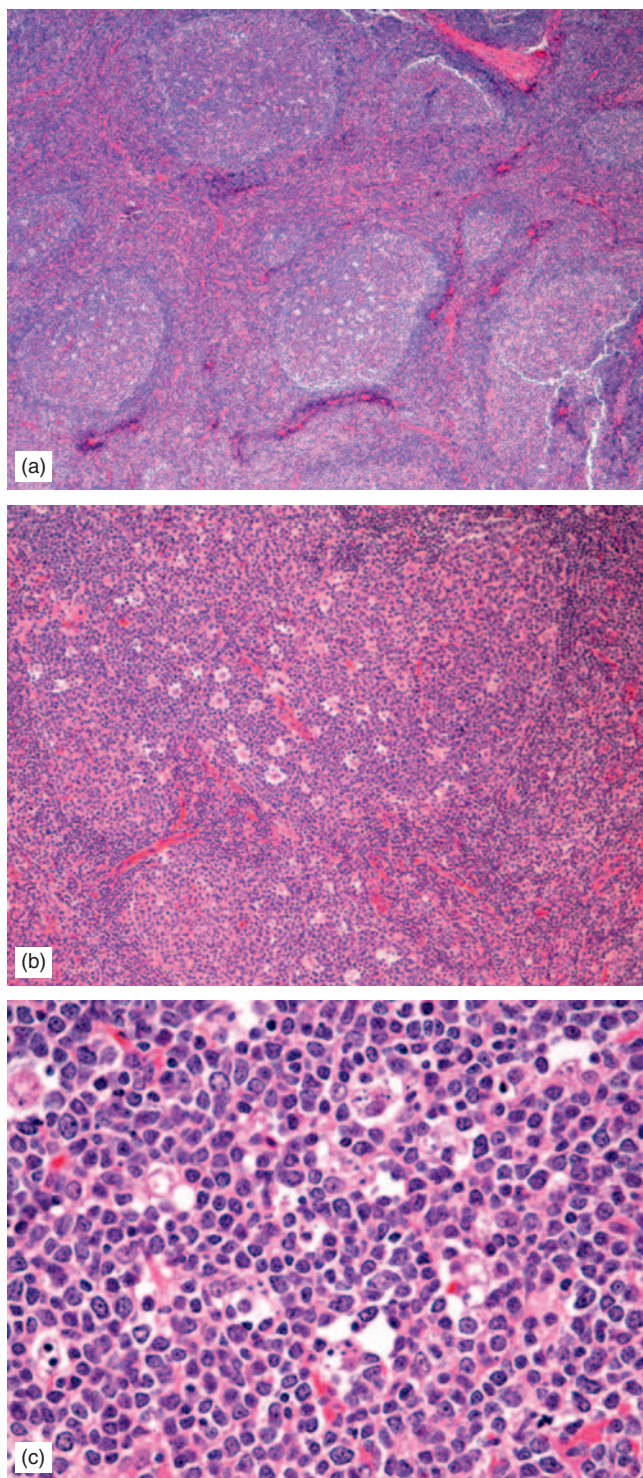


FIGURE 6.3 Follicular hyperplasia in a patient with rheumatoid arthritis: (a) low power, (b) intermediate power, and (c) high power.

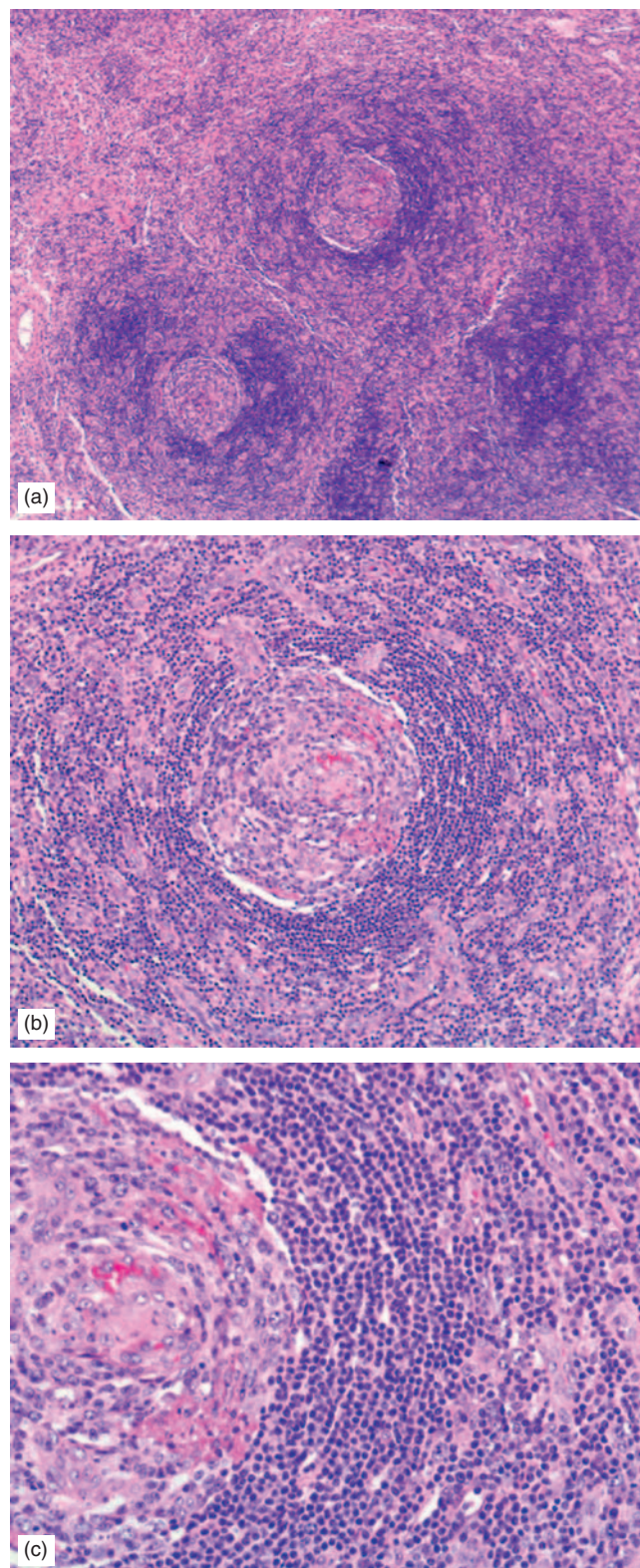


FIGURE 6.4 Castleman's disease, hyaline vascular type, demonstrating follicular hyperplasia and expansion of mantle zones demonstrating an "onion skin" pattern: (a) low power, (b) intermediate power, and (c) high power.

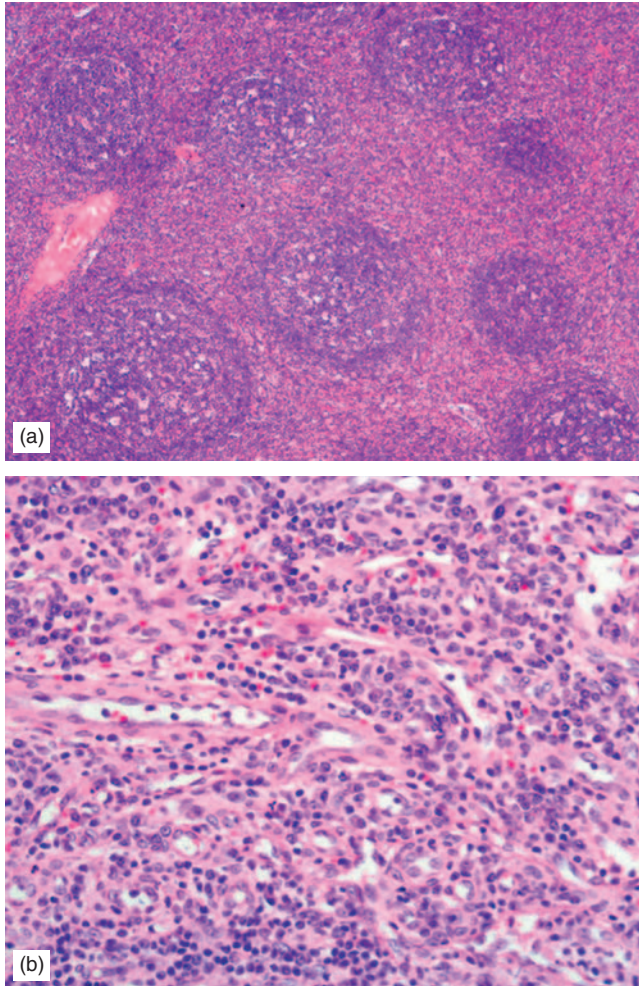


FIGURE 6.5 Lymph node biopsy section from a patient with Castleman's disease, hyaline vascular type, demonstrating follicular hyperplasia (a) and vascular proliferation in the interfollicular areas (b).

with carbon pigments. Sinus histiocytosis with pigments may be seen in the regional lymph nodes of tattooed areas. Melanin-containing histiocytes are seen in dermatopathic lymphadenitis (see later). Sinus histiocytosis is also noted in lysosomal storage diseases, hemophagocytosis (often with the presence of hemosiderin pigments), and post-lymphangiography [20, 21]. Histiocytes express CD68 and are strongly positive for lysozyme.

Sinus Histiocytosis with Massive Lymphadenopathy

Sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease) is a bilateral, painless, massive cervical lymphadenopathy reported in patients under the age of 20 years [22–26]. An association with human herpes virus 6 has been reported in some cases [27]. The affected lymph nodes show a characteristic feature consisting of sheets of large sinus histiocytes with abundant clear or foamy cytoplasm containing numerous lymphocytes (or less frequently

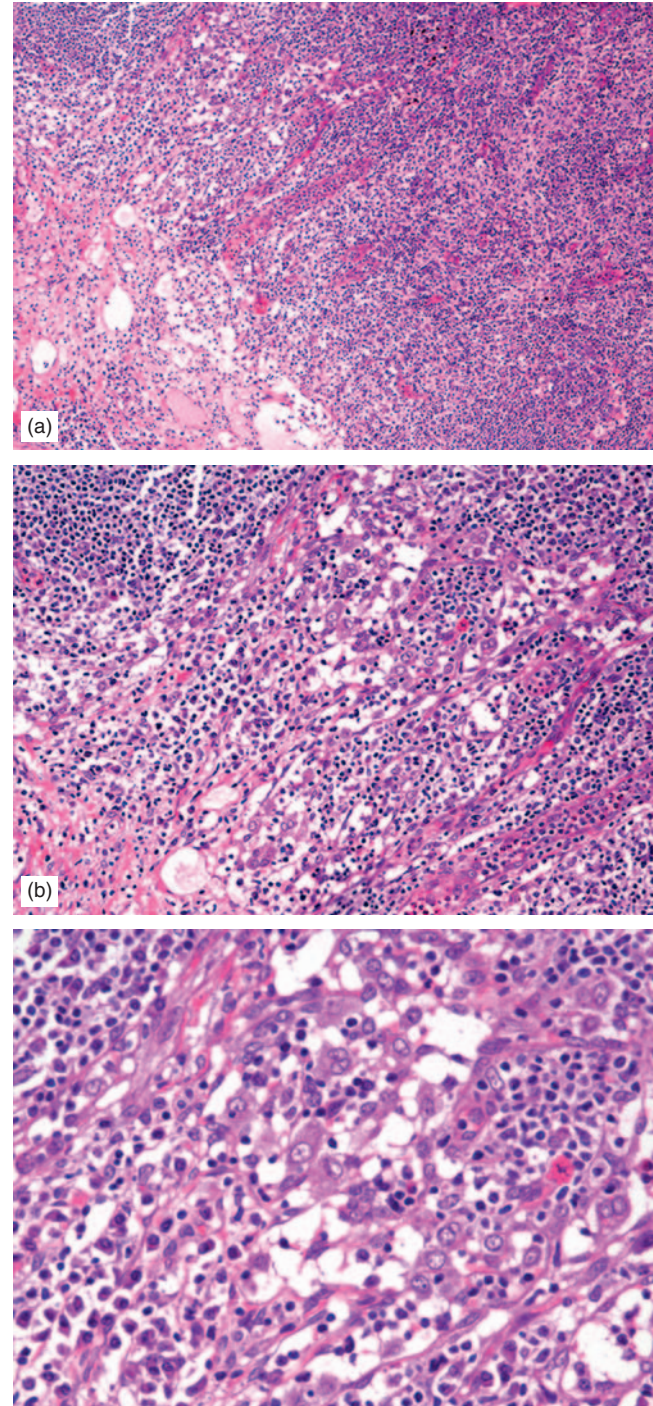


FIGURE 6.6 Sinus histiocytosis. Lymph node biopsy section demonstrating dilatation of the sinusoids and presence of numerous histiocytes: (a) low power, (b) intermediate power, and (c) high power.

other hematopoietic cells) (Figure 6.7). These lymphocytes appear intact. The active penetration of cells into and through larger cells is called emperipolesis. Often, there is a marked thickening of the capsule with pericapsular fibrosis. Histiocytes express CD68 and are strongly positive for lysozyme [28].

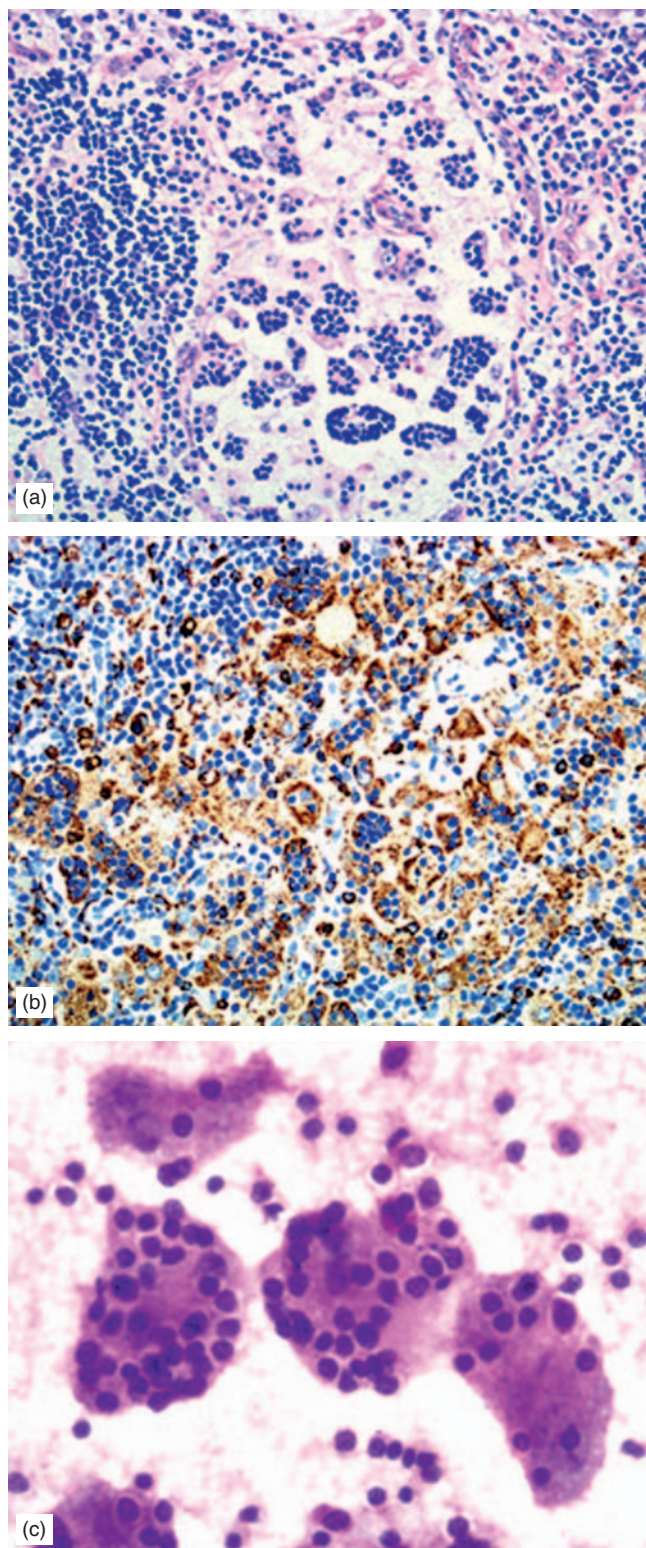


FIGURE 6.7 Sinus histiocytosis with massive lymphadenopathy (Rosai–Dorfman disease). Dilated sinuses contain numerous lymphocyte-containing histiocytes. Histiocytes demonstrate emperipolesis with the presence of numerous intact lymphocytes. (a) H&E section, (b) immunohistochemical stain for CD68, and (c) touch preparation. Courtesy of Sophie Song, M.D., Ph. D., Department of Pathology and Laboratory Medicine, UCLA Medical Center.

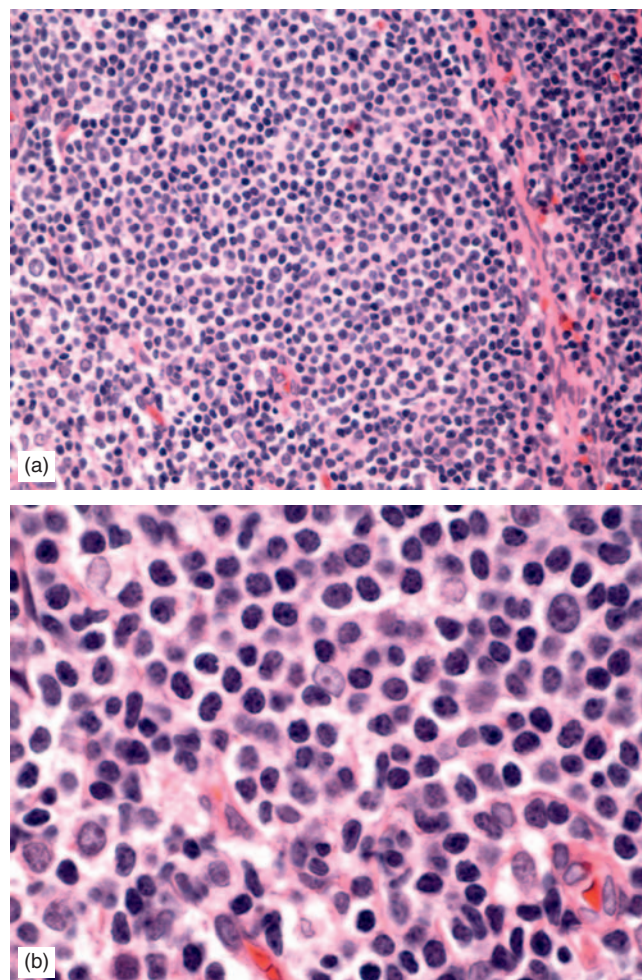


FIGURE 6.8 Lymph node section demonstrating hyperplasia of monocytoid B-cells: (a) low power and (b) high power.

Monocytoid B-Cell Hyperplasia

Hyperplasia of monocytoid B-cells (marginal zone B-cells) is predominantly sinusoidal, but it may also extend to the paracortical areas (Figure 6.8) [1, 29, 30]. Monocytoid B-cell hyperplasia has been observed in toxoplasmosis, viral infections, cat-scratch disease, and other non-specific lymphoid hyperplasias [1, 2, 31]. It is characterized by the presence of clusters of medium to large cells with abundant pale cytoplasm, round or convoluted nuclei, moderately condensed chromatin, and inconspicuous nucleoli. These cells express B-cell-associated antigens and lack monocytic markers. Monocytoid B-cell hyperplasia is often associated with follicular hyperplasia. The differential diagnosis includes monocytic/histiocytic disorders, marginal zone B-cell lymphoma, hairy cell leukemia, and mastocytosis.

Whipple's Disease

Whipple's disease is a multi-system bacterial infection caused by *Tropheryma whippeli*, most frequently involving the gastrointestinal tract and mesenteric lymph nodes

[1, 2, 32, 33]. The affected lymph nodes show sinus histiocytosis. Histiocytes show vacuolated cytoplasm and contain PAS-positive sickle-form particles. *Tropheryma whippeli* can be detected by the polymerase chain reaction (PCR) technique using species-specific sequences of the 16S ribosomal RNA [34].

Langerhans Cell Histiocytosis

Langerhans cell histiocytosis frequently affects lymph nodes in a sinus pattern (see Chapter 21 for more details) [35–37]. Langerhans cells are large mononuclear cells with abundant cytoplasm, folded or grooved nuclei, and inconspicuous nucleoli. They express CD1, S100, and Langerin (CD207), and demonstrate Birbeck granules in their cytoplasm by electron microscopy.

Hemophagocytic Syndromes

Hemophagocytic syndromes are discussed in Chapter 21 [38–40]. Affected lymph nodes show sinus histiocytosis with evidence of hemophagocytic activities.

GRANULOMATOUS LYMPHADENITIS

Granulomas may appear in different morphologic configuration, such as clusters of epithelioid histiocytes (e.g. sarcoidosis, fungal infections), with caseous necrosis (tuberculosis), or suppurative granulomas (e.g. cat-scratch disease, lymphogranuloma venereum). Sarcoidosis and cat-scratch disease are briefly discussed later as examples of this category.

Sarcoidosis

Sarcoidosis is a multicentric granulomatous disorder of unknown etiology characterized by well-defined epithelioid granulomas, often surrounded by lymphocytes and plasma cells, and less frequently by fibrosis (Figure 6.9) [1, 2, 41, 42]. Multinucleated giant cells may be present and may show asteroid bodies. Granulomas are usually non-necrotizing but occasionally may show small central fibrinoid necrosis. Sarcoid-like granulomas may be present in lymph nodes of patients with Hodgkin lymphoma, Crohn's disease, Whipple's disease, fungal infections, or tuberculosis. Therefore, morphologic diagnosis of sarcoidosis is based on the exclusion of other granulomatous lesions. Mediastinal and pulmonary hilar lymph nodes are the most frequent sites of involvements.

Cat-Scratch Disease

Cat-scratch disease is caused by a small, gram-negative bacterium, *Bartonella henselae*.

It is characterized by a variable-sized central fibrinoid necrosis, which in early stages is suppurative and contains

variable numbers of neutrophils. The central necrosis is surrounded by palisading histiocytes, creating a stellate granuloma (Figure 6.10) [1, 2, 43–45]. The Warthin-Starry silver stain facilitates the detection of the bacteria. The involved lymph nodes often show follicular hyperplasia, sinus histiocytosis, proliferation of monocytoid B-cells, and increased number of immunoblasts.

Most of the cases are found in patients under the age of 18 years, and axillary lymph nodes are the most frequent sites of involvement. A history of exposure to cats is found in almost all cases [1, 2, 45].

MIXED PATTERN

Certain types of reactive lymphadenitis demonstrate a combination of follicular, paracortical, and/or sinus patterns. Examples are dermatopathic lymphadenitis, toxoplasmosis, Kikuchi's disease, and Kimura's disease.

Dermatopathic Lymphadenitis

Dermatopathic lymphadenitis is a reactive process often associated with inflammatory skin disorders [1, 2, 46]. The involved lymph nodes show nodular expansion of paracortical areas with pale-staining large cells consisting of histiocytes and Langerhans cells (Figures 6.11 and 6.12) [1, 2, 47]. Some of the histiocytes may contain melanin or hemosiderin pigments. Histiocytes are strongly CD68 and lysozyme positive and Langerhans cells express CD1 and CD100 (Figure 6.12) [48]. The nodular areas also contain scattered lymphocytes and plasma cells. The affected lymph nodes show sinus histiocytosis. Patients with mycosis fungoides may show regional enlarged lymph nodes with morphologic features very similar to dermatopathic lymphadenitis. Therefore, detection of nodal involvement in early stages of mycosis fungoides without significant architectural effacement is very difficult and may require gene rearrangement studies [49].

Toxoplasmosis

The involved lymph nodes in toxoplasmosis show follicular hyperplasia with expanded germinal centers. Multiple clusters of epithelioid histiocytes are present in the paracortical areas, adjacent to the follicles and within the germinal centers (Figure 6.13). Multinucleated giant cells are usually not present [50–52]. The subcapsular and trabecular sinuses are dilated and may contain numerous monocytoid B-cells. Similar morphologic features have been reported in enlarged lymph nodes of HIV-infected patients.

Kikuchi's Disease

Kikuchi's disease is a necrotizing lymphadenitis affecting young adults of particularly Asian descent. Women

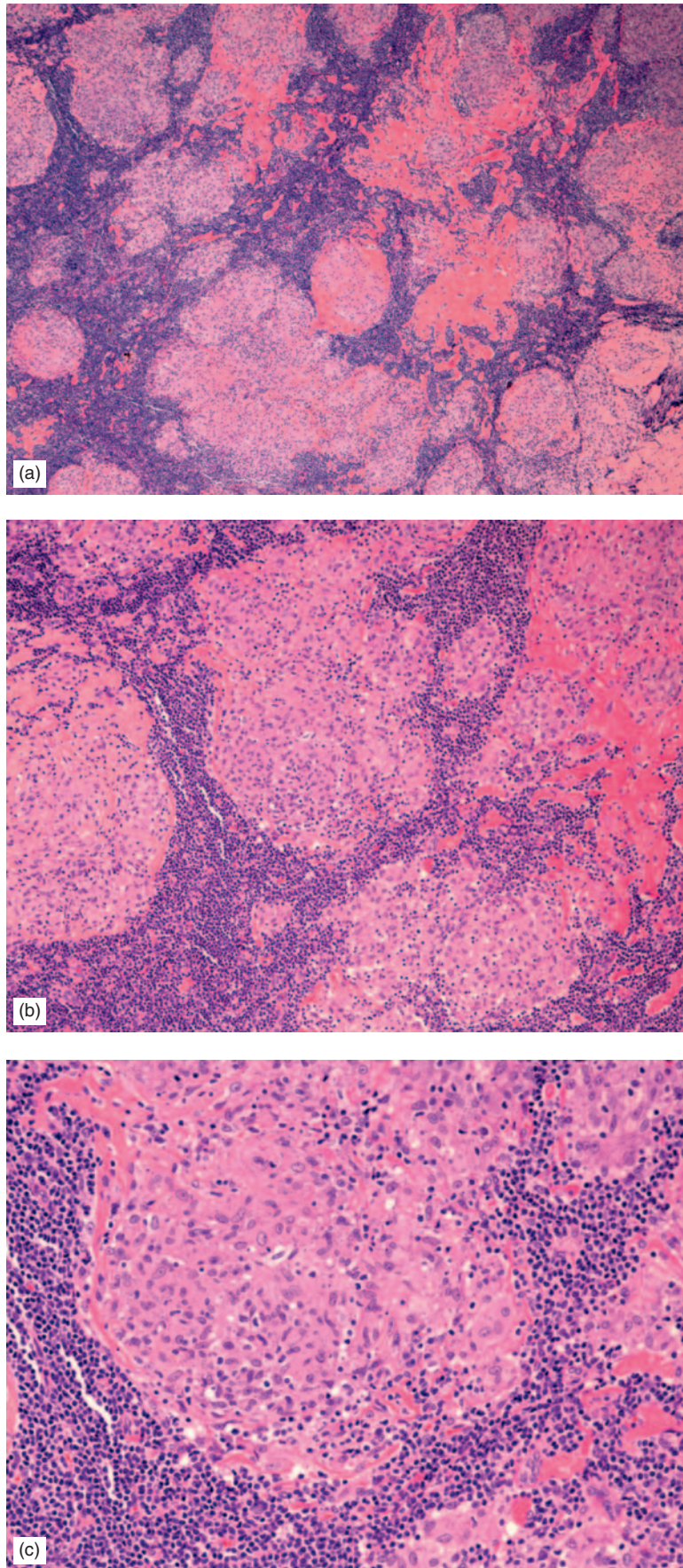


FIGURE 6.9 Lymph node: clusters of epithelioid histiocytes in a patient with sarcoidosis (a, b, and c) low, intermediate, and high power views, respectively.

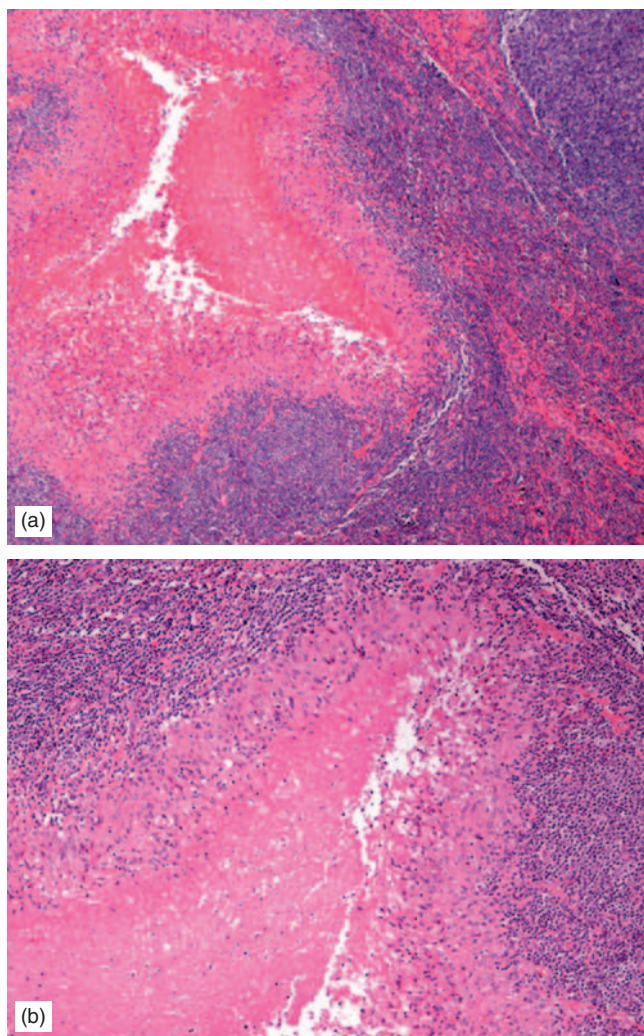


FIGURE 6.10 Lymphadenitis with irregular areas of necrosis surrounded by palisading histiocytes (stellate granulomas) are characteristic features of cat-scratch disease: (a) low power and (b) high power.

are affected more than men [53–57]. It is a self-limited localized lymphadenitis, often involving cervical lymph nodes. The characteristic morphologic findings are patchy areas of necrosis with prominent karyorrhexis (apoptosis) and nuclear debris with or without coagulative necrosis. Neutrophils are rare or absent. The apoptotic foci are predominantly located in cortical and paracortical areas and are surrounded by large histiocytes and immunoblasts. These cells show some atypical features and may resemble Hodgkin cells. Some phagocytic histiocytes may show eccentric nuclei (signet ring histiocytes) and others may show foamy cytoplasm. Follicular hyperplasia may be present. Differential diagnosis of Kikuchi's disease includes Hodgkin lymphoma, systemic lupus erythematosus, and herpes simplex lymphadenitis (Figure 6.14) [58].

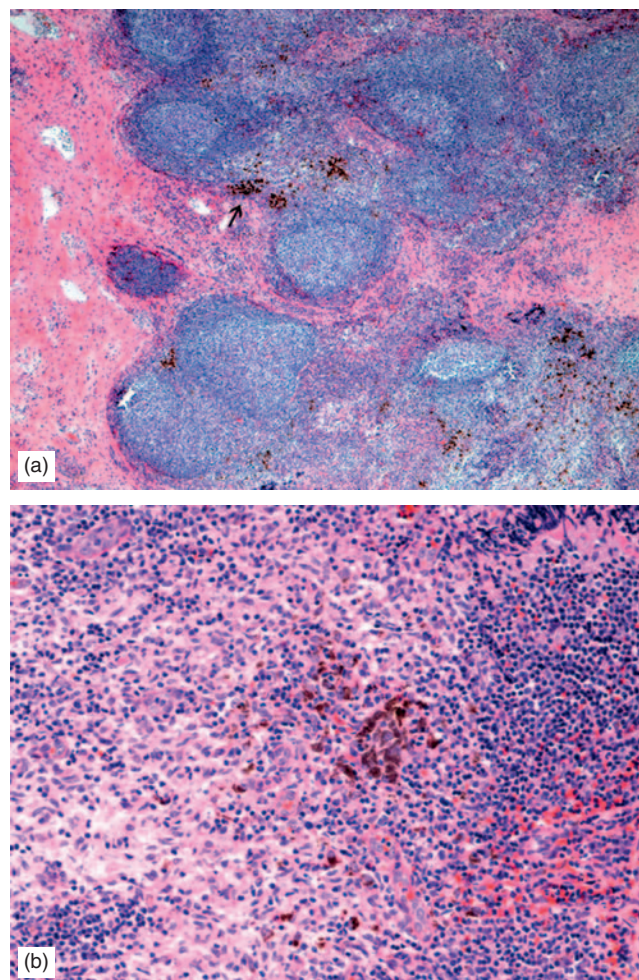


FIGURE 6.11 Dermatopathic lymphadenitis demonstrating follicular hyperplasia, sinus histiocytosis, pigmentation, and nodular expansion of paracortical areas with pale-staining large cells consisting of histiocytes and Langerhans cells: (a) low power and (b) high power.

Kimura's Disease

Kimura's disease (eosinophilic hyperplastic lymphogranuloma) is a rare form of chronic inflammatory disorder involving subcutaneous tissue, predominantly in the head and neck region. It is frequently associated with regional lymphadenopathy and/or salivary gland involvement [59]. This condition has a predilection for males of Asian descent [59–61]. The involved lymph nodes show follicular hyperplasia with the presence of numerous eosinophils in the follicle, paracortex, sinusoids, and perinodal soft tissues [2]. There is a distinct IgE-positive dendritic network in the germinal centers [2].

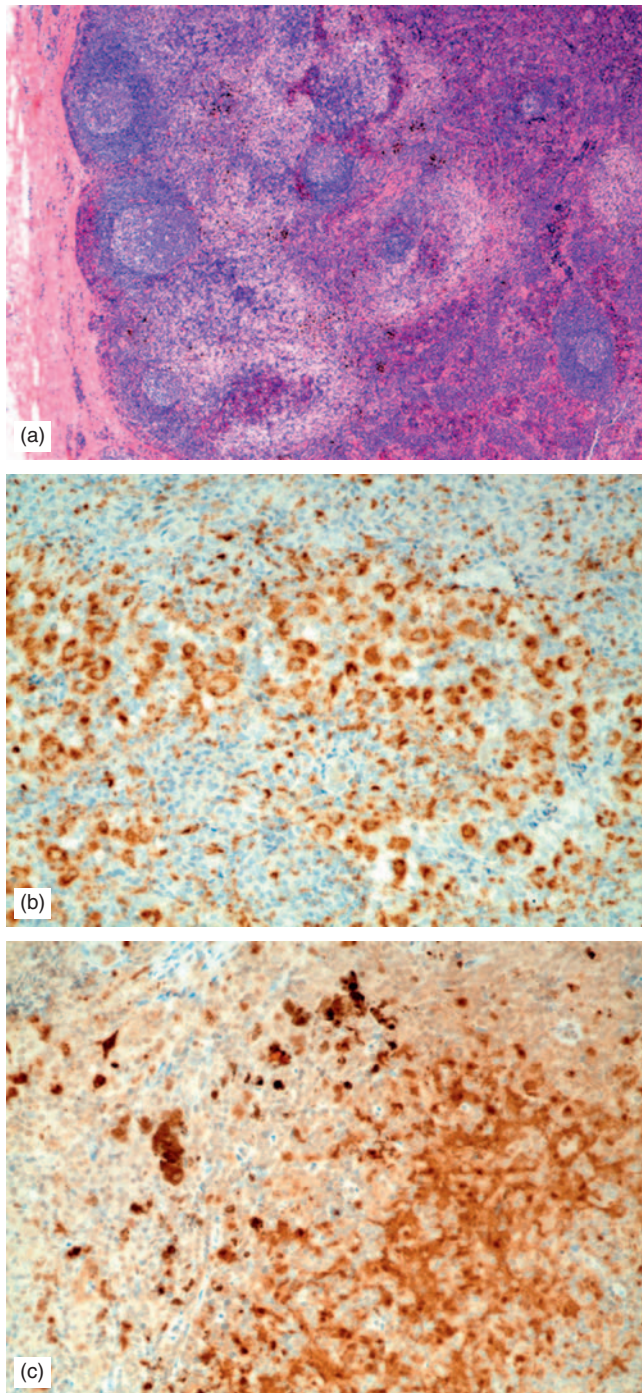


FIGURE 6.12 Dermatopathic lymphadenitis demonstrating nodular expansion of paracortical areas with pale-staining large cells (a). These cells consist of a mixture of Langerhans cells (b), S100-positive, and histiocytes (c), CD68-positive.

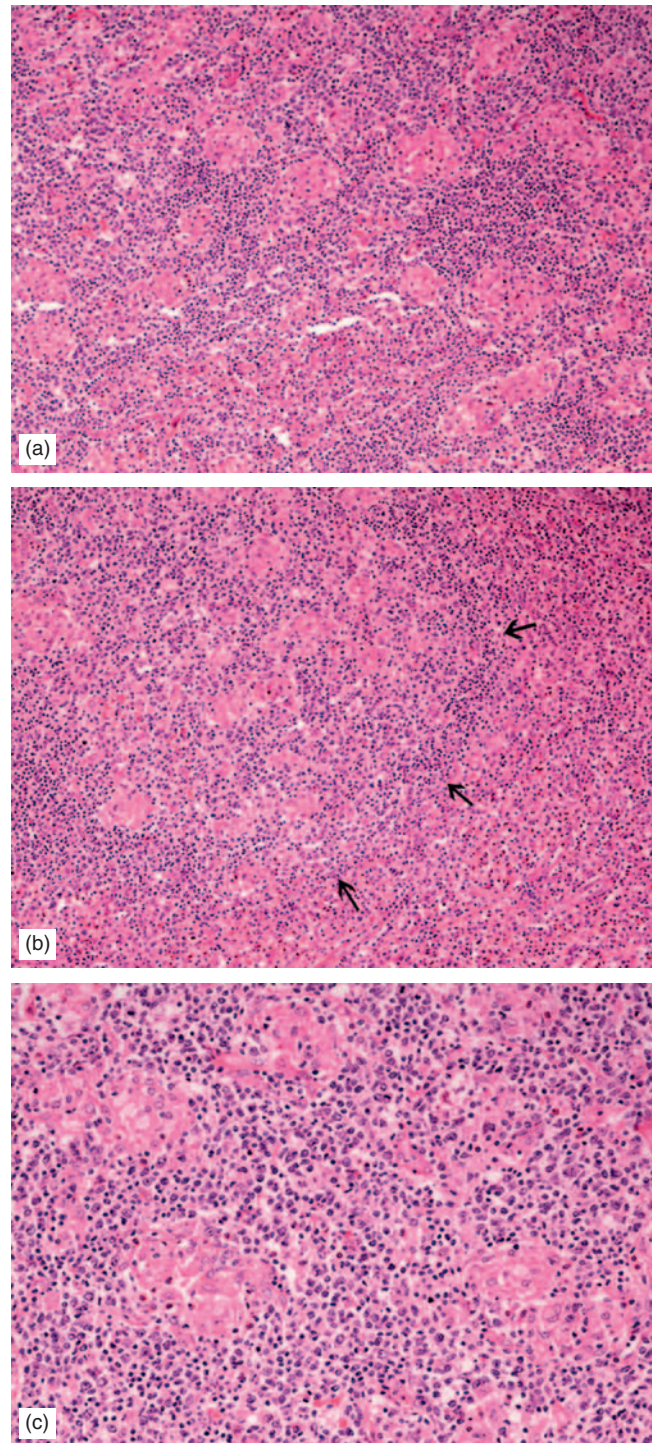


FIGURE 6.13 Epithelioid granulomas in toxoplasmosis. Clusters of epithelioid histiocytes are present in the paracortical areas (a) as well as within the follicles (b, arrows). A higher power view of the epithelioid granulomas is presented in (c).

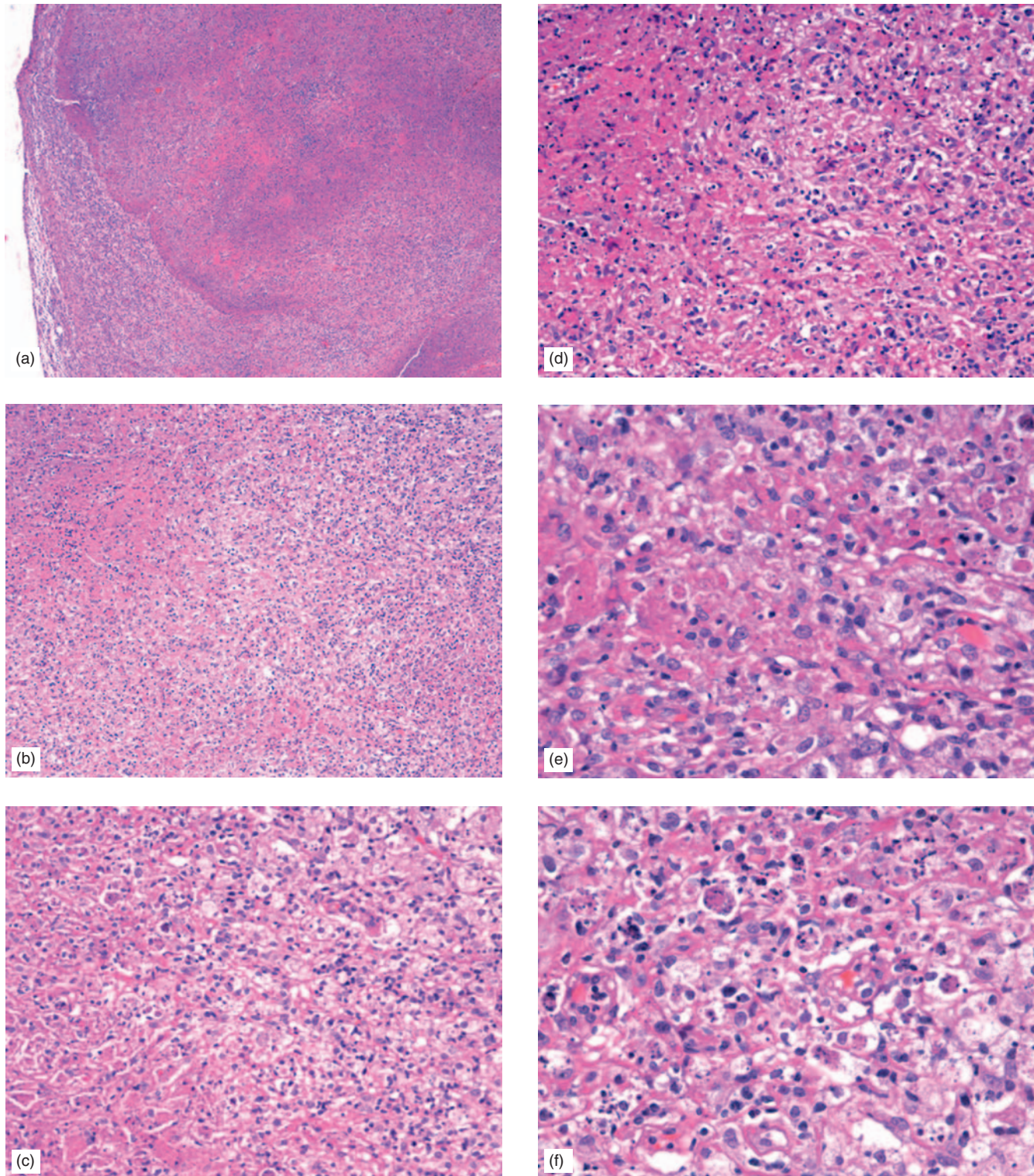


FIGURE 6.14 Kikuchi's disease. A large area of necrotic debris and histiocytes replacing a significant proportion of the lymph node: (a) low power, (b) intermediate power, and (c) high power views. The necrotic debris lack neutrophils (c and d). Numerous histiocytes show pale cytoplasm and contain nuclear debris (e and f). Courtesy of Sophie Song, M.D., Ph.D., Department of Pathology and Laboratory Medicine, UCLA Medical Center.

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Bone Marrow Aplasia

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Bone marrow aplasia (aplastic anemia) refers to those hematologic conditions that are caused by a marked reduction and/or defect in the pluripotent or committed stem cells, or the failure of the bone marrow microenvironment to support hematopoiesis. The clinical outcome is anemia, leukopenia, and thrombocytopenia (pancytopenia) [1–4]. The term “aplastic anemia” (AA) is a misnomer, because the patients, in addition to anemia, also suffer from leukopenia and thrombocytopenia.

In this chapter, constitutional and acquired AA, dyskeratosis congenita, Shwachman–Diamond syndrome, Diamond–Blackfan anemia (DBA), amegakaryocytosis, and paroxysmal nocturnal hemoglobinuria are discussed (Table 7.1) [5–9]. Bone marrow failure due to myelodysplastic syndromes (MDS), leukemias, myelofibrosis, and other disorders are discussed in the following chapters.

FANCONI ANEMIA

Fanconi anemia (FA) is the most common form of congenital bone marrow aplasia [10–12]. It is an autosomal recessive or X-linked disorder with a prevalence of about 1 in 300,000 in most populations, but with much higher frequencies in the Afrikaner population of South Africa and Ashkenazi Jews [10, 12, 13]. FA is associated with physical abnormalities and affects males more than females with a ratio of about 2:1 [10, 12]. The congenital AA without physical abnormalities is known as Eastern–Dameshek anemia.

Etiology and Pathogenesis

At least 11 complementation groups (FA-A to FA-L) have been reported, and so far, eight genes

TABLE 7.1 Classification of bone marrow aplasia.

- | |
|---|
| 1. Constitutional <ul style="list-style-type: none"> (a) Fanconi anemia (b) Dyskeratosis congenita (c) Shwachman–Diamond syndrome (d) Diamond–Blackfan anemia (e) Amegakaryocytosis |
| 2. Acquired <ul style="list-style-type: none"> (a) Idiopathic aplastic anemia (b) Secondary aplastic anemia <ul style="list-style-type: none"> (i) Chemical and physical agents <ul style="list-style-type: none"> – Drugs and other chemicals – Radiation (ii) Infection <ul style="list-style-type: none"> – Viral: hepatitis, EBV, HIV – Others: tuberculosis, dengue fever (iii) Immunologic (humoral and/or cellular) (iv) Metabolic (pancreatitis, pregnancy) |
| 3. Paroxysmal nocturnal hemoglobinuria |
| 4. Others <ul style="list-style-type: none"> (a) Hypoplastic myelodysplastic syndromes (b) Bone marrow replacement <ul style="list-style-type: none"> (i) Malignant neoplasms (ii) Fibrosis (iii) Others |

have been cloned [10, 12, 14–16]. The FA genes may contribute to genomic stability, DNA repair, and control of apoptosis [10, 12, 14–16]. The defects in DNA repair in FA patients may lead to spontaneous chromosomal breakage, which is significantly enhanced in homozygotes, particularly when the drugs mitomycin and diepoxybutane are used [10, 14–16]. The *FA-A* gene is responsible for 60–65% of the patients with FA and has been mapped to chromosome 16q24.3

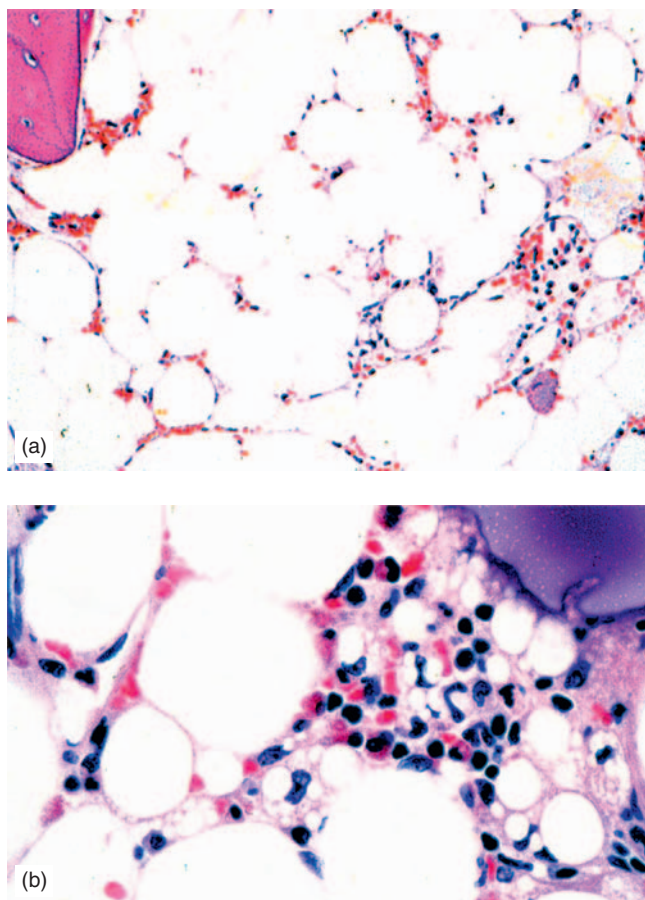


FIGURE 7.1 Bone marrow biopsy section from a patient with Fanconi anemia demonstrating marked hypocellularity with small foci of hematopoietic cells: (a) low power and (b) high power views.

[10, 14–16]. Interestingly, the *FAD1* gene turned out to be the same as the *BRCA2* gene involved in familial breast/ovarian cancer, a dominantly inherited cancer predisposition [11, 17, 18].

An abnormality in the interferon signaling mechanism has been suggested in FA which may lead to enhanced apoptosis. There is also some evidence of abnormal telomeres in FA patients [19]. Telomeres have been implicated in the control of both genomic stability and cell proliferation capacity [19].

Pathology

Morphology

Bone marrow biopsy sections in early stages of the disease may appear hyper- or normocellular with some megaloblastic changes but eventually become hypoplastic and depict marked hypocellularity with scattered foci of hematopoietic cells, predominantly erythroid (Figure 7.1) [1–3]. Often there is an increased proportion of plasma cells and lymphocytes. Bone marrow smears may show increased mast cells and

evidence of hemophagocytosis, particularly in early stages of the disease. These morphologic features are not pathognomonic for FA and are also observed in patients with acquired AA [1–3].

Blood examination is usually normal at birth. Usually, microcytosis is the first detected abnormality, followed by elevated levels of fetal hemoglobin, thrombocytopenia, and neutropenia between the ages of 5 and 10 years [1–3, 12].

Molecular Genetics and Cytogenetics

Although the central diagnostic study for FA is the demonstration of the chromosome breakage defect, the rearrangements are so varied and inconsistent that the molecular study of the breakpoints has no practical diagnostic value [20]. However, the cloning of the genes associated with most of the complementation groups has made definitive molecular genetic diagnosis possible. DNA testing of several of the FA genes is now available, consisting of either gene sequencing or targeted mutation analysis such as the predominant IVS4 + 1A→T mutation in the Ashkenazi-Jewish population. Moreover, once the precise gene defect is discovered in a proband, the same approach can be used for prenatal diagnosis in future pregnancies within the family. In addition, population-based carrier screening (i.e. for those couples with no family history of the disorder) is being offered in some centers for Ashkenazi Jews of reproductive age, in whom the carrier frequency for mutations in the gene for FA type C is 1 in 90 [21].

It is worth noting that FA is the first disorder for which couples have availed themselves of the technique of preimplantation genetic diagnosis (involving single-cell biopsy and polymerase chain reaction (PCR) testing of early embryos conceived by *in vitro* fertilization) to select a baby having the identical HLA type to serve as a bone marrow donor for their living affected child [22]. Clearly, such efforts, although technically feasible, raise a number of troubling ethical issues [23, 24].

Clinical Aspects

Characteristic congenital malformations, such as generalized skin hyperpigmentation (café au lait spots) and areas of hypopigmentation, microcephaly, hypogonadism, abnormality of thumbs, and short stature, are present in up to 60–70% of the affected children [6, 10, 12]. Other abnormalities include microphthalmia, renal hypoplasia, horseshoe kidneys, or double urethras [6, 10, 12]. The hematologic findings evolve gradually and may take months to years to reach full-blown pancytopenia. Thrombocytopenia is among the most common initial findings [10, 12, 25].

FA patients have an increased risk of developing clonal bone marrow cytogenetic abnormalities, such as myelodysplastic syndrome (MDS) and/or acute myelogenous leukemia (AML) [26–28]. The actuarial risk of MDS and AML is over 50% by the age of 40. This risk is higher in patients with cytogenetic abnormalities. There is also an elevated risk of squamous carcinoma of head and neck and gynecologic system, and various other solid tumors, in patients with FA [26, 29].

OTHER CONGENITAL BONE MARROW APLASIAS

Dyskeratosis Congenita

Dyskeratosis congenita (DC) is an X-linked recessive trait which is characterized by bone marrow failure and a triad of mucosal leukoplakia, nail dystrophy, and abnormal skin pigmentation [12, 30–32]. Approximately 20% of the patients may also suffer pulmonary dysfunction characterized by reduced diffusion capacity [33].

The dyskeratin gene (*DKC1*) at chromosome Xq28 is mutated. This gene appears to play a role in ribosome synthesis and telomerase function [34]. Sequence analysis of the *DKC1* gene is available in a small number of laboratories. Detection of mutations can be used for diagnosis, carrier screening, and prenatal testing.

The approximate median ages for the demonstration of somatic abnormalities and bone marrow failure are 8 and 10 years, respectively [12, 33]. Bone marrow becomes markedly hypoplastic with morphologic features similar to those of FA.

Over 90% of the affected patients are male [12, 33, 35]. DC patients have a higher tendency to develop MDS, AML, and skin and oropharynx cancer [35, 36].

Shwachman–Diamond Syndrome

Shwachman–Diamond syndrome or Shwachman–Diamond–Oski syndrome is a rare autosomal disorder which presents its clinical symptoms during infancy [37, 38]. It is characterized by skeletal anomalies, short stature, pancreatic insufficiency, and progressive bone marrow failure [37, 38]. The pancreatic insufficiency in this syndrome is distinguished from cystic fibrosis by a normal sweat chloride test result. Mutations of a gene referred to as Shwachman–Bodian–Diamond syndrome (*SBDS*) have been reported; it is located on chromosome 7q11 and so far has no known function [39]. Sequencing of the *SBDS* gene is available in several reference laboratories [40]. Although exocrine pancreatic dysfunction is a feature of the disorder and the cystic fibrosis gene (*CFTR*) is located on chromosome 7 (distal at 7q31.2), the pathogenesis is different and these patients do not have cystic fibrosis, so *CFTR* mutation testing is of no value. Over-expression of *p53* has been observed in bone marrow [41], but this is a non-specific finding and not used for diagnostic purposes.

Neutropenia is the major hematologic characteristics of this disorder, which is often intermittent or cyclic (Figure 7.2). Patients are prone to infection, particularly caused by gram-negative organisms, *Hemophilus influenza* or *Staphylococcus aureus*. Elevated levels of fetal hemoglobin are detected in up to 80%, and AA is observed in 20–25% of patients with Shwachman–Diamond syndrome [38, 42].

Diamond–Blackfan Anemia

Diamond–Blackfan anemia (DBA) is a pure red cell aplasia predominantly demonstrated in infancy and early childhood (Figure 7.3) [42–44]. DBA is about 45% familial and is

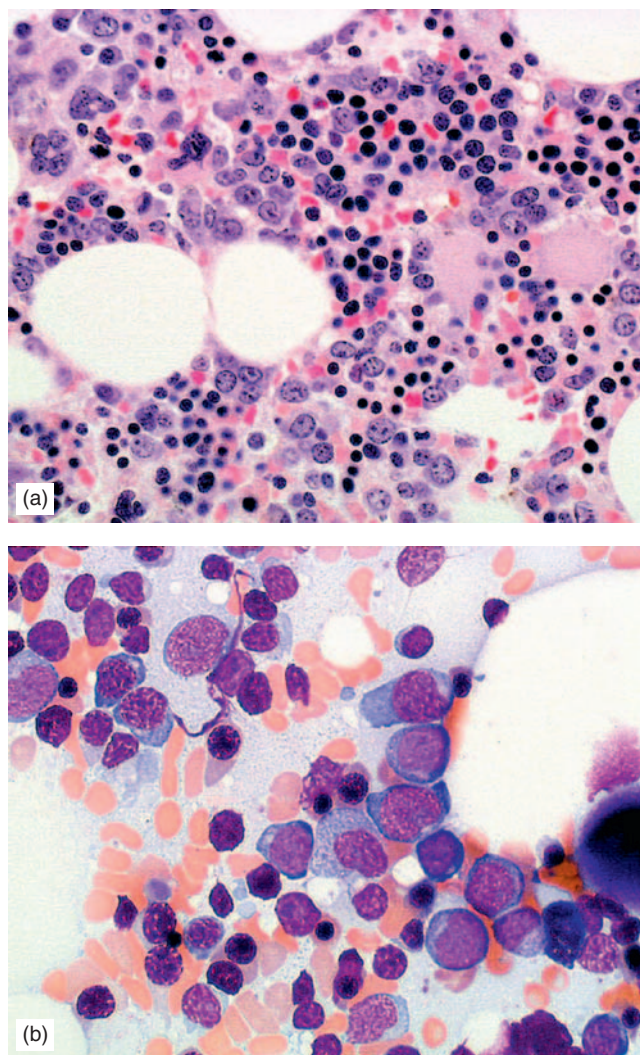


FIGURE 7.2 Bone marrow smears of patients with Shwachman–Diamond syndrome show reduced number of neutrophils and bands: (a) low power and (b) high power.

often associated with physical anomalies, such as thumb malformations, growth retardation, and craniofacial deformities [42, 45]. Hematologic findings include macrocytic anemia, elevated fetal hemoglobin levels, and increased erythrocyte adenosine deaminase activity [42, 43]. DBA patients may eventually develop pancytopenia and aplastic bone marrow.

The first DBA gene, *RPS19*, located on chromosome 19q13, was found to be mutated in approximately 25% of patients. This gene encodes a protein which is a component of the 40S ribosomal subunit. Most patients are found to be heterozygous suggesting that the disease is caused by protein haploinsufficiency [46]. There are probably at least two other genes associated with some cases of DBA, but they have not been defined sufficiently for testing. The genetics of the disorder are complicated by the absence of family history in many cases, which could be due to either sporadic incidence or a dominant gene with low penetrance. Testing for parvovirus B19 by PCR of bone marrow samples may be performed as part of the differential diagnosis of red cell aplasia in an infant.

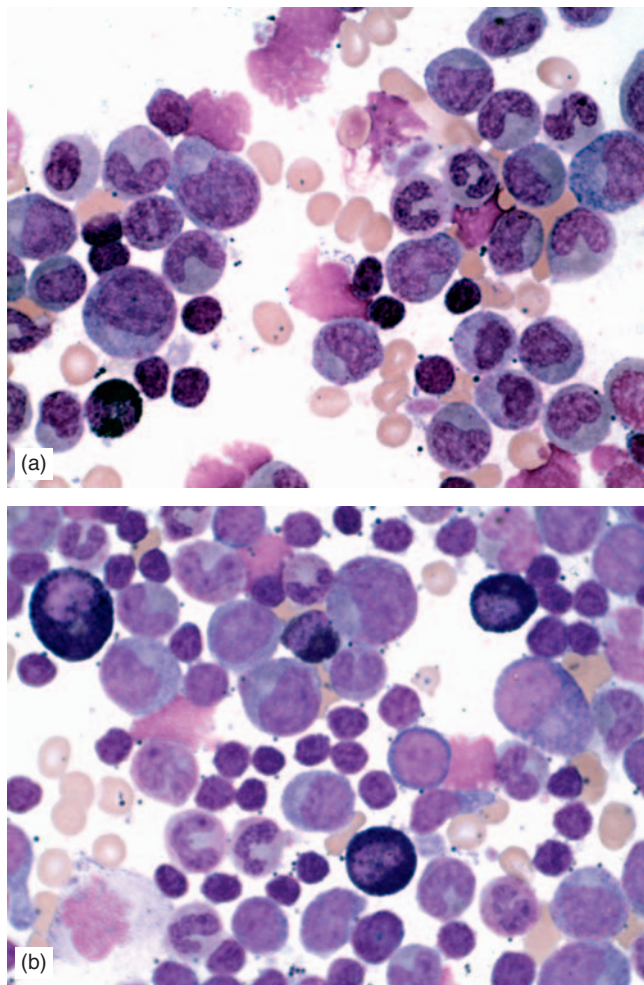


FIGURE 7.3 Bone marrow smears from a patient with pure red cell aplasia (Diamond–Blackfan anemia) demonstrating lack of erythroid precursors: (a) low power and (b) high power.

Amegakaryocytosis

Congenital amegakaryocytosis (amegakaryocytic thrombocytopenia) is a rare disorder of infancy with markedly reduced or absent megakaryocytes in bone marrow and therefore isolated thrombocytopenia [12, 47]. Approximately 50% of these patients may eventually develop AA and pancytopenia. The cause of this disorder in some children appears to be mutations of the thrombopoietin receptor gene, *MPL* (CD110) on chromosome 1p35 [48]. The autosomal recessive form of amegakaryocytosis is caused by mutations in the *MPL* gene [49], which block end-organ response to thrombopoietin (see Chapter 24). Because of the rarity of the disorder, testing for these mutations is available only on a research basis.

The serum concentration of thrombopoietin is elevated. Clinical symptoms include bleeding into the mucous membranes, gastrointestinal tract, and skin. Absence of radial bones is observed in the majority of the patients (thrombocytopenia with absent radius, TAR syndrome) [47, 50].

ACQUIRED APLASTIC ANEMIA

Acquired AA is characterized by severe bone marrow hypocellularity and pancytopenia. The term “acquired” refers to non-congenital causative mechanisms which could be immunologic, environmental, or unknown. Patients often have a history of exposure to a wide spectrum of chemical and physical agents and various diseases. However, since there is daily exposure to unlimited and widespread chemicals, such as insecticides, fertilizers, food additives, and herbal medicine, the exact causative factor(s) is not detected in about 50–75% of AA patients [51–53]. Therefore, acquired AA is divided into two major categories: (1) idiopathic AA (with no known etiology) and (2) secondary AA.

Etiology and Pathogenesis

The etiology of AA in most patients is still not clear (idiopathic). Several studies support the destruction or suppression of bone marrow stem cells by immune mechanisms as the major contributing factors [53–55]. These studies include:

1. Positive effects of various immunosuppressive agents such as antithymocyte globulin (ATG), cyclosporine, cyclophosphamide, or corticosteroids on the clinical outcome of the patients with AA [56–58].
2. Occurrence of AA in association with graft versus host disease following allogeneic bone marrow transplantation [51].
3. Association of AA with immunologic disorders such as eosinophilic fasciitis and Grave’s disease [59, 60].
4. Evidence of clonal expansion of CD8-positive T-cells in patients with idiopathic AA [61, 62].
5. Evidence of a reduction in the bone marrow natural killer cells of patients with AA [63].
6. Lymphocyte activation by antigens, chemicals, or viruses may suppress hematopoiesis through the release of interferon gamma [64, 65].

In certain conditions damage to bone marrow stem cells and/or microenvironment is caused by agents that are considered relatively harmless. This could be due to the presence of several factors, such as severely depleted marrow stem cells, excessive acquired vulnerability of the hematopoietic precursors or stromal cells, or development of autoimmunity.

Many drugs, such as chloramphenicol, felbamate, nifedipine, gold, sulfonamides, and phenylbutazone, may play a role in the development of AA (Table 7.2) [51, 66–70]. The bone marrow suppression effect of these drugs is often reversible, meaning that by discontinuation of the medication the bone marrow activities eventually come back to normal. Of these drugs, chloramphenicol is perhaps the best documented one, as it was widely used in the United States between 1948 and 1967 [71]. The toxic effects of chloramphenicol are associated with vacuolization of the erythroid precursors, presence of ringed sideroblasts (accumulation of iron in mitochondria)

TABLE 7.2 Drugs associated with aplastic anemia.

1. Anti-inflammatory drugs and analgesics
(a) Butazones
(b) Diclofenac
(c) Gold
(d) Indomethacin
(e) Piroxicam
2. Antibiotics
(a) Chloramphenicol
(b) Isoniazid
(c) Penicillin
(d) Streptomycin
(e) Sulfonamides
(f) Tetracycline
3. Anti-epileptic
(a) Felbamate
(b) Methionine
(c) Methsuximide
(d) Phenacemide
(e) Troxidone
4. Anti-diabetic
(a) Chlorpropamide
(b) Tolbutamide
5. Anti-malarial
(a) Mepacrine
(b) Chloroquine
(c) Piramethamine
6. Others
(a) Allopurinol
(b) Chlorpromazine
(c) Nifedipine
(d) Organic arsenicals

and increased serum iron. Occasionally, the toxic effects are irreversible, leading to a sustained AA [53].

Development of AA has been reported in association with the exposure to a garden variety of non-pharmacological chemicals, such as organic solvents, pesticides, and aniline dyes [72, 73]. There are reports of development of AA as a consequence of use of traditional herbal medications [74]. Benzene metabolites suppress DNA synthesis and inhibit proliferation of hematopoietic precursors leading to AA, MDS, and AML [75].

Radiation may induce AA by impairing hematopoietic stem cells (HSCs) and damaging the bone marrow microenvironment. Lethal or sub-lethal amounts of total body irradiation, long-term continuous exposure to small amounts of radiation, and high doses of local therapeutic radiation have all been considered as possible contributors to the development of AA [51, 74].

Certain viruses are able to damage stem cells and/or bone marrow microenvironment and cause AA [53, 76–78]. Hepatitis-associated AA is observed 2–3 months after the onset of acute hepatitis, though the responsible virus has not been identified yet. Between 2% and 5% of patients

with AA show evidence of hepatitis [53, 76, 77]. Other viruses such as HIV, EBV, rubella virus, and parvovirus B19 may also cause AA [76, 78].

Reports of higher frequency of HLA-DR2 and HLA-B14 in patients with AA suggest a genetic predisposition [79, 80]. Also, there is a close relationship between AA and paroxysmal nocturnal hemoglobinuria (see later), which is considered a clonal stem cell disorder.

Pathology

Morphology and Laboratory Findings

The diagnosis of AA is established by bone marrow examination [1–3]. Bone marrow is markedly hypocellular with a very high proportion of fatty tissue and stromal cells (Figure 7.4). All hematopoietic elements are decreased but are morphologically normal. There is no evidence of a malignant infiltrate or diffuse fibrosis. Peripheral blood shows pancytopenia with reduced reticulocyte count. A severe AA is defined as [81]:

1. A bone marrow cellularity of <25% of normal cellularity for age in biopsy sections, or
2. A bone marrow cellularity of <50% of normal cellularity for age with <30% hematopoietic cells, plus at least two of the following:
 - (a) Absolute erythrocyte count of <40,000/ μ L.
 - (b) Absolute neutrophil count <500/ μ L.
 - (c) Platelet count <20,000/ μ L.

When the criteria for severe AA are met and the absolute neutrophil count is <200/ μ L, the patient is considered to have a very severe AA [53].

The hypocellular bone marrow biopsy sections show scattered islands of hematopoietic cells randomly distributed throughout the marrow. These islands are predominantly erythroid and contain very few megakaryocytes. Bone marrow smears consist predominantly of adipocytes and stromal tissue with scattered hematopoietic cells. Occasionally, some of the aspirated smears may show cellular marrow particles, giving the wrong impression of a normocellular or even hypercellular marrow. For this reason, bone marrow biopsies are preferred for the establishment of the diagnosis of AA [1–3]. Some bone marrow smears may show increased proportion of lymphocytes, plasma cells, macrophages, and mast cells. These cells either appear as well-defined aggregates or are diffusely dispersed in the stroma. There may be evidence of hemophagocytosis, particularly in early stages of the disease.

Peripheral blood examination reveals pancytopenia. Anemia is usually normochromic and normocytic, but macrocytosis and anisocytosis may be present. The reticulocyte count is low, and platelets, neutrophils, monocytes, eosinophils, and basophils are reduced. Neutrophils may show toxic granulation. The lymphocyte count is normal or low.

Molecular Genetics and Cytogenetics

Owing to its heterogeneous and non-genetic etiology, there are no specific molecular tests for acquired AA. Mutation testing of genes associated with the hereditary disorders, and PCR-based detection of implicated viruses, may be

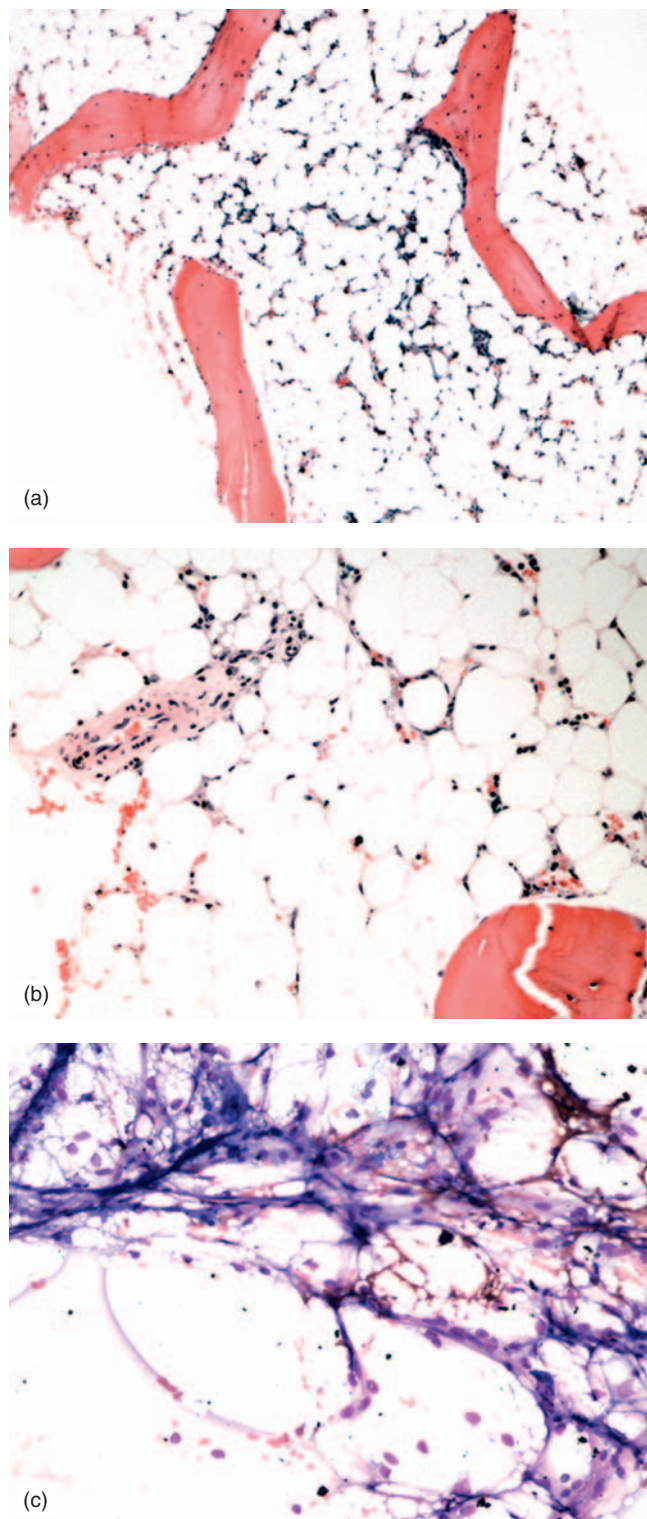


FIGURE 7.4 Bone marrow biopsy sections: (a) low power and (b) high power, from a patient with AA demonstrating marked hypocellularity. The bone marrow smear shows fatty tissue and stromal cells (c).

performed as part of the differential diagnostic work-up. Approximately 4% of patients with AA show cytogenetic abnormalities, such as 5q⁻, monosomy 7, and trisomy 6 or 8 (Figures 7.5 and 7.6) [82, 83].

Clinical Aspects

The incidence of AA is significantly higher (about fivefold) in the Far East than the West [84, 85]. Clinical manifestations of AA are non-specific and are usually related to pancytopenia. Pallor, fatigue, purpura and mucosal hemorrhage, and recurrent infections are common findings [51, 53]. Occasionally cardiopulmonary symptoms associated with severe anemia are the presenting clinical picture. The cause of death is usually infection, particularly disseminated fungal forms.

The outcome of untreated severe AA is very poor, with over 70% death rate within 1 year. Prognosis is also age-dependent, with better outcome in patients under 49 years than those over 60 years [51, 53, 86, 87]. The treatment of choice under the age of 45 is HSC transplantation [88, 89]. The major problem is lack of HLA-matched donors. Only 25–30% of AA patients find proper donors. Immunosuppressive therapy is recommended for patients over the age of 45. Immunosuppressive agents include ATG, corticosteroids, and cyclosporine [56, 90]. Hematopoietic growth factors, such as G-CSF, have been added to the immunosuppressive regimen with some beneficial effects.

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired stem cell disorder associated with a defect in cell membrane glycosyl phosphatidylinositol (GPI) anchor due to mutation of the *PIG-A* gene. This defect leads to partial or complete loss of certain GPI-linked membrane proteins, such as CD14, CD16, CD24, CD48, CD52, CD55, CD58, CD59, CD66, and CD73 (Table 7.3) [91–96]. Some of these proteins, such as CD55 and CD59, play an inhibitory role in the activation of the complement system, and therefore their absence leads to complement-induced lysis and hemolytic anemia [95–97]. CD55, also known as decay accelerating factor (DAF), is expressed by all hematopoietic cells and is an inhibitor of C3 and C5 convertases [95–97]. Similarly, CD59 is expressed by all hematopoietic cells. It is referred to as membrane inhibitor of reactive lysis (MIRL) and binds to C8 component of the complement system and prevents polymerization of the complement components [95–98]. PNH is characterized by hemolytic anemia (often with hemoglobinuria), venous thrombosis, and bone marrow failure [91–94].

Etiology and Pathogenesis

The fundamental pathogenic process in PNH is the defect in the production of GPI anchor [91, 94, 97, 98]. This defect is due to a somatic mutation in the phosphatidylinositol glycan complementation class A (*PIG-A*) gene at the level of HSC. So far, constitutional *PIG-A* defects have not been described. The *PIG-A* gene is located on the short arm of the X chromosome [91, 94, 99]. Patients with PNH have one or more stem cell GPI-deficient clones. More than one hundred different mutations have been reported

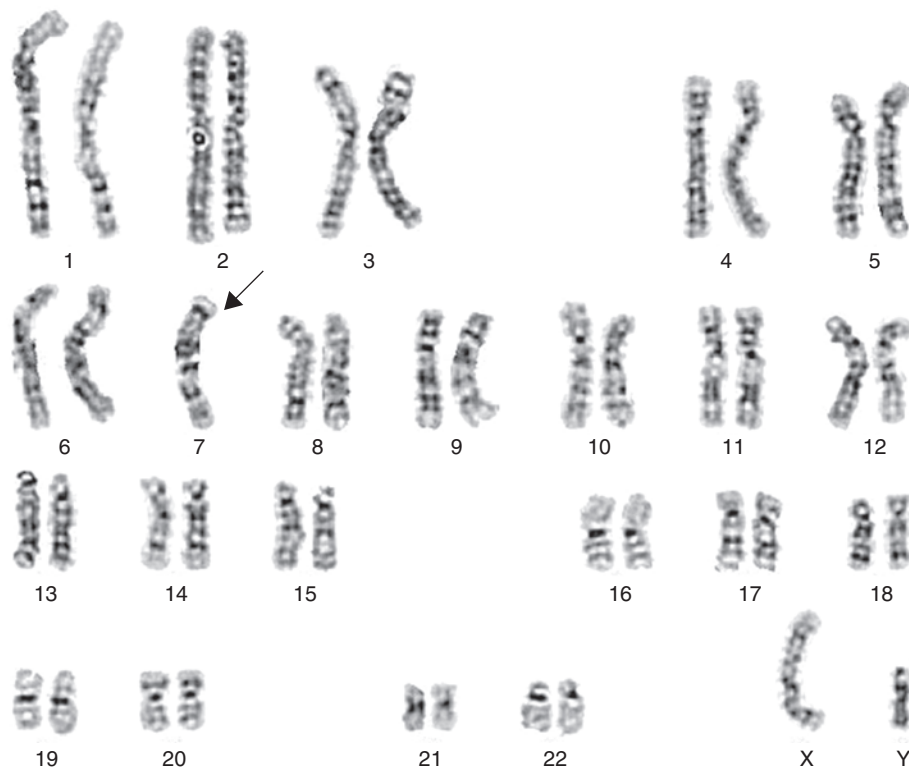


FIGURE 7.5 A G-banded karyotype showing monosomy 7 (arrow) in a patient with AA.

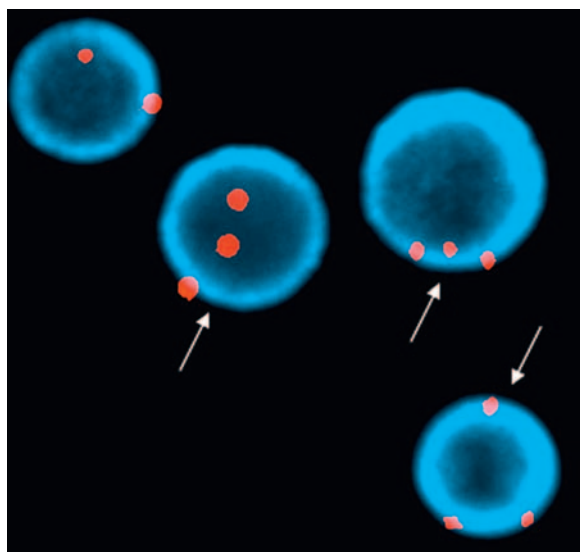


FIGURE 7.6 FISH studies for chromosome 8 in interphase. Cells demonstrating trisomy 8 (arrows).

[91, 99]. However, the defect in GPI production may not be sufficient enough for the manifestation of PNH, since *PIG-A* mutations have been described in the stem cells of normal individuals as well. It has also been shown that most patients with AA and refractory anemia who carry HLA-DRBI have an expanded population of GPI-deficient clones [85, 94, 95, 100, 101]. However, in most instances, the GPI-deficient clones found in AA and refractory anemia are generally small.

It seems that other hematopoietic alterations are necessary to allow the *PIG-A* deficient clone to dominate. For example, in some patients development of AA precedes PNH [91, 101, 102]. The dominant pathogenic theory for the development of PNH in patients with AA is that the development of AA suppresses the GPI-defective stem cells less than the normal stem cells, and therefore, when the degree of bone marrow suppression is reduced by therapy (such as ATG or cyclosporine), the GPI-defective clone has a better chance to emerge. An autoimmune process may play a role in the pathogenesis of PNH. The involved auto reactive T-cells may selectively damage the GPI-positive HSCs, whereas GPI-negative HSCs would escape damage and be able to proliferate [91, 101, 102].

Pathology

Morphology and Laboratory Findings

Bone marrow in most instances is markedly hypocellular and presents morphologic features similar to AA (Figure 7.7) [1–3]. However, some patients may show normo- or even hypercellular marrow. There is often erythroid preponderance. Stainable iron is usually absent, primarily due to loss of iron secondary to hemoglobinuria and hemosiderinuria [1–3].

Blood examination commonly reveals severe anemia with some degree of granulocytopenia and thrombocytopenia [1–3, 91, 92]. There is evidence of intravascular hemolysis by the presence of hemoglobinuria, hemosiderinuria and elevated reticulocyte count. There is a reduction in plasma haptoglobin levels and an increase in plasma lactate

TABLE 7.3 Some of the GPI-linked proteins deficient in paroxysmal nocturnal hemoglobinuria*.

Molecule	CD	Comments
Complement Regulatory Molecules		
DAF	CD55	Decay accelerating factor
MIRL	CD59	Membrane inhibitor of reactive lysis
Enzymes		
Ecto-5'-nucleotidase	CD73	Lymphocytes
ADP-ribosyl transferase	CD157	T-cells and neutrophils
Adhesion Molecules		
Blast-1	CD48	Leukocytes; binds CD24
LFA-3	CD58	All hematopoietic cells
Adhesion molecule 1	CD66a	Granulocytes, epithelium
NCA-95	CD66b	Granulocytes
NCA-50/90	CD66c	Granulocytes, epithelium
Carcinoembryonic antigen	CD66e	Epithelium
Others		
NA1/NA2	CD16	Neutrophils and natural killer cells
Campath-1	CD52	Lymphocytes and monocytes
BA-1	CD24	B-cells and granulocytes
Thy-1	CD90	Stem cell subset, T-cell subset

* Adapted from Hall C, Richards SJ, Hillmen P. (2002). The glycosylphosphatidylinositol anchor and paroxysmal nocturnal haemoglobinuria/aplasia model. *Acta Haematol* **108**, 219–30.

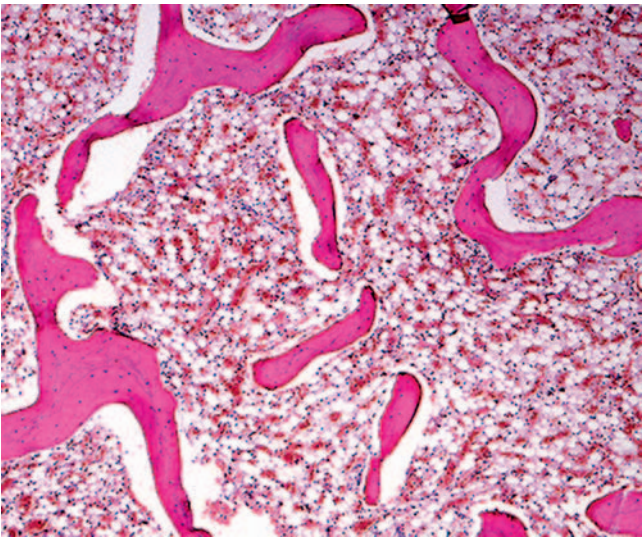


FIGURE 7.7 Bone marrow biopsy section from a patient with PNH demonstrating marked hypocellularity.

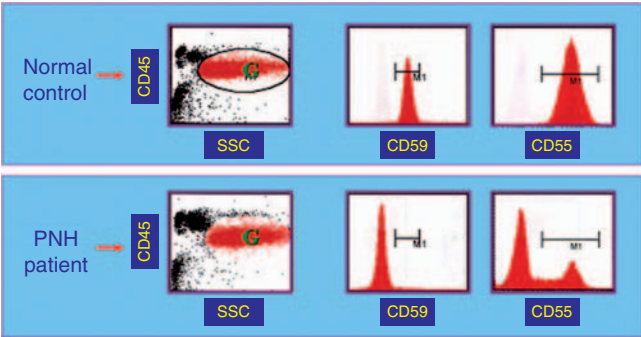


FIGURE 7.8 Flow cytometry comparison of CD55 and CD59 expressions on the granulocytes of a normal control and a patient with PNH. Granulocytes of the normal control show expression of CD55 and CD59, whereas in the PNH patient there is lack of expression of CD55 and CD59 expression is partially lost.

dehydrogenase (LDH) level [91–93]. The leukocyte alkaline phosphatase score is reduced.

For years, the diagnosis of PNH was based on the sensitivity of the red cells to lysis by complement. This was determined by the sucrose lysis screening test and the confirmatory Ham acid hemolysis test [91–93]. In the sucrose lysis test, the patient's red cells are incubated with serially diluted isotonic sucrose solutions. Under these conditions the complement system is activated and the test is considered positive if there is evidence of hemolysis. In the Ham test, the pH of serum is reduced to activate the complement system and to induce hemolysis in the PNH red cells. However, nowadays, immunophenotyping by flow cytometry (see the following section) is considered the standard procedure because of the higher sensitivity and specificity it provides.

Flow Cytometry

Study of the expression of GPI-linked proteins on the hematopoietic cells by flow cytometry is now the recommended approach in the diagnosis of PNH [103–106]. Complete or partial loss of these proteins is indicative of GPI deficiency. There is a garden variety of GPI-linked proteins to choose for different types of hematopoietic cells, and therefore there are a number of options regarding antibody selection, cell type, and gating strategies. Most laboratories include at least two GPI-linked proteins, often CD55 and CD59, for each cell type under study. Red cells and granulocytes are the most frequent cell types studied (Figure 7.8) [103–106]. A transmembrane antigen is also used as a positive control. Each cell type is divided into three categories according to the status of the protein expression:

1. Type I: cells with normal expression
2. Type II: cells with partial absence
3. Type III: cells with complete absence.

PNH patients show a significant proportion (usually over 10%) of the type II and/or type III cells.

Analysis of red cells: Monoclonal antibodies against CD55 and CD59 are used for the analysis of the red cells. The red cell population is gated by forward scatter (FSC) and side scatter (SSC) amplification in log mode, and the

purity of red cells is checked by the percent glycophorin A-positive cells in the gated population. The red cell analysis is clearest in untransfused patients [103–106]. A marked variation in the distribution of types I, II, and III is observed from patient to patient, though sometimes the separation of subtypes is not clear-cut. The type III red cells appear to have a significantly shorter survival (about 17–60 days) than type I red cells. Red cells are suitable for testing up to 3 weeks if kept at 4°C.

Analysis of granulocytes: Anti-CD55 and CD59 monoclonal antibodies are often used for granulocyte analysis. The granulocyte population is gated by FSC and SSC characteristics, but some laboratories use a myeloid-associated marker, such as CD15 or CD33, to optimize the detection of granulocytes. Antibodies against other GPI-linked proteins, such as CD16 and CD66, could also be utilized [103–106]. However, since CD16 is not expressed by normal eosinophils, its use is not recommended for the samples with a high proportion of eosinophilia [102–106]. Flow cytometric studies on granulocytes should be carried out within the first few hours following collection [102–106].

Analysis of monocytes: Although red cells and granulocytes are the most frequent cell types used for the diagnosis of PNH, several investigators have also analyzed peripheral blood monocytes for this purpose. Anti-CD14 and CD55 monoclonal antibodies are the favorite ones, though some laboratories have looked at the expression of CD48 and CD59 [102–106]. Analysis of the monocytes is accomplished by the use of FSC and SSC properties or CD45 expression and SSC properties.

Analysis of lymphocytes: Although lymphocytes, similar to other hematopoietic cells, show loss of GPI-linked proteins, they are infrequently used for the establishment of PNH diagnosis. The expression of CD55 and CD59 is variable on normal lymphocytes, and therefore these two markers may not provide reliable information in PNH flow cytometry studies. CD48 appears to be a more suitable marker, because it provides a clearer separation between normal and PNH lymphocytes [103–106]. Immunophenotypic studies of B-cells in PNH show that in the majority of patients with active PNH, B-cells are comprised primarily of naïve cells characterized with a CD27⁺ IgG⁺ IgM⁺ and IgD⁺ phenotype [107].

Analysis of platelets: Expression of CD55 and CD59 has been studied on platelets in PNH patients [103–106, 108]. Platelets are usually gated based on their FSC and SSC properties and the use of non-GPI-linked CD molecules CD41 and/or CD61 as platelet markers. However, these studies are infrequent and their diagnostic significance has not been established. Frequent thromboembolic events in PNH strongly suggest an abnormal platelet function in these patients, probably due to the deficiency of CD55 and CD59. But, so far, no definitive platelet defect has been characterized and the underlying mechanisms remain unclear. However, a recent report indicates a profound platelet hyporeactivity in PNH patients based on clot formation, adhesion, and aggregation assays, concluding that the venous thromboembolism is probably induced by activation and dysregulation of plasma coagulation factors [91–96].

In general, peripheral blood is the sample of choice for immunophenotypic studies in PNH. Red cells and granulocytes are the recommended cell types, and CD55 and

CD59 are the most commonly used markers for these studies. Samples with recent history of blood transfusions may provide ambiguous results. The following important points are provided by Richards and associates in their review article regarding flow cytometric studies in PNH [103–106]:

1. A small proportion of patients (about 5%) may display only a granulocyte PNH clone.
2. The chance of the detection of a red cell PNH clone is reduced after a severe episode of hemolysis.
3. In patients with severely hypoplastic marrow, there may be insufficient numbers of granulocytes for analysis.
4. In red cell analysis, deficiencies of at least two GPI-linked antigens are required to establish a PNH diagnosis, since congenital non-PNH single-antigen deficiencies have been observed rarely.

Molecular Genetics and Cytogenetics

As described earlier, the cornerstone of PNH diagnosis rests on the flow cytometry findings. Reported mutations in the *PIG-A* gene on chromosome Xp22 are numerous and heterogeneous, and further complicated by the presence of a pseudogene on chromosome 12. DNA sequencing is not generally available on a clinical basis. It must be kept in mind that the gene mutations found in PNH are acquired, not inherited, so they will only be found in the abnormal clone [14, 109]. Cytogenetic abnormalities in paroxysmal nocturnal hemoglobinuria usually occur in hematopoietic cells that are glycosylphosphatidylinositol-anchored protein (GPI-AP) positive [110]. Various chromosomal aberrations have been reported in up to 24% of patients with FA including trisomy 5, trisomy 6, trisomy 8, and monosomy 7 [111, 112].

Clinical Aspects

The disease may affect patients at any age, but the peak incidence is between 20 and 35 years [91, 92]. The severity of the clinical findings varies considerably from patient to patient and may include any of the following manifestations [91, 92, 95, 96]:

1. Acquired intravascular hemolysis demonstrated by hemoglobinemia, hemoglobinuria, hemosideriuria, and negative direct antiglobulin (Coombs') test. Plasma haptoglobin level is low and plasma LDH level is often elevated.
2. Thrombosis of the relatively large veins in odd places, such as hepatic (Budd–Chiari syndrome), mesenteric, portal, or cerebral veins. The rate of venous thrombosis in western countries approaches 40% and is the major cause of death in PNH patients. Arterial thrombosis is rare.
3. Bone marrow hypoplasia leading to pancytopenia. As briefly mentioned earlier, in a significant proportion of PNH patients (up to 30%), there is a previous history of AA [101].

The possible association between PNH, MDS, and acute myeloid leukemia has been the subject of several case reports, although the incidence is relatively low at around 5% [92,

TABLE 7.4 Differential diagnoses in bone marrow aplasia.

Disorder	Bone marrow morphology	Immunophenotype	Cytogenetics and molecular genetics
Constitutional aplasias	Normo- to hypercellular at early stages, hypocellular marrow at later stages	Non-contributory	Frequent chromosomal breakage, sometimes -7, mutations in causative genes
Acquired AA	Hypocellular marrow	Often increased cytotoxic T-cells, strong association with HLA-DR2	Sometimes 5q-, -7, +6, +8, viral PCR
PNH	Hypocellular marrow	Loss of GPI-linked proteins, such as CD55 and CD59	Mutations in <i>PIG-A</i> gene
Hypocellular MDS	Hypocellular marrow with significant dysplastic changes, and sometimes increased blasts	Abnormal phenotypic patterns sometimes increased CD34+ and/or CD117+ cells	-7, +8, 5q-, 20q-, and other chromosomal aberrations
Hypoplastic AML	Hypocellular marrow with $\geq 20\%$ blasts	Increased CD45 ^{dim} + cells expressing myeloid markers, often CD34 and/or CD117	Frequent chromosomal aberrations involving 11q, 16q, or t(15;17), t(8;11), t(9;22), and others
Hypocellular hairy cell leukemia	Hypocellular marrow with the presence of hairy cells and often evidence of fibrosis	TRAP+, CD103+, CD25+, CD22+, 11c+	Not known

113–115]. Therapeutic approaches include iron and folic acid supplementation, red blood cell transfusion, and treatment with prednisone and androgen derivatives [92, 94]. Anticoagulation therapy is used for episodes of thrombosis. HSC transplantation has been used in selected cases [92, 116].

DIFFERENTIAL DIAGNOSIS

Morphologic features of bone marrow in advanced stages of constitutional marrow aplasias, acquired AA, and PNH are indistinguishable. Also, other bone marrow lesions, such as hypocellular MDS, hypoplastic AML, and hypocellular hairy cell leukemia, may morphologically mimic AA (Table 7.4). Clinical history and information regarding other clinicopathologic parameters are imperative for accurate diagnosis. It is important to remember that a proportion of patients with constitutional or acquired AA may eventually develop MDS or AML.

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Myelodysplastic Syndromes

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Myelodysplastic syndromes (MDS) are a group of hematologic disorders distinguished by clonal expansion of defective hematopoietic stem cells leading to abnormal maturation and peripheral blood cytopenia. These disorders are also known as refractory anemias (RA), dysmyelopoietic syndromes, and were originally labeled by some investigators as preleukemias. The peripheral cytopenias may be demonstrated as anemia, thrombocytopenia, granulocytopenia, or pancytopenia. Some categories of MDS show increased bone marrow blast cells and have a higher chance to eventually transform to acute myeloid leukemias (AMLs). The overall transformation rate to acute leukemia depends on the subtype of MDS, the presence or absence of chromosomal aberrations, and types of these abnormalities.

Classification of MDS by the World Health Organization (WHO) includes the following categories [1–5a] (Table 8.1):

- Refractory anemia (RA)
- Refractory anemia with ringed sideroblasts (RARS)
- Refractory cytopenia with multilineage dysplasia (RCMD)
- Refractory cytopenia with multilineage dysplasia and ringed sideroblasts (RCMD-RS)
- Refractory anemia with excess blasts (RAEB)
- MDS associated with isolated del(5q)
- MDS unclassifiable.

In a recently revised draft of the WHO classification, *myelodysplastic syndromes in children* was added to the list (updated WHO classification in press [5b]).

The WHO classification represents the clonal forms of MDS. However, non-clonal myelodysplastic changes have been observed in a variety of conditions, such as severe inflammatory states, viral infections, autoimmune disorders, megaloblastic anemia, exposure to arsenic, status post-chemotherapy, and endocrine dysfunctions.

ETIOLOGY AND PATHOGENESIS

There are two major categories of MDS: primary (with no known cause) and secondary (usually post-treatment chemotherapy or irradiation). The etiology and pathogenesis of the primary MDS are not clearly understood. Some familial clustering has been reported, but no causative germline mutations have been identified. Clonality of the underlying marrow failure has been supported by various molecular and cytogenetic techniques, such as karyotyping, X-chromosome inactivation studies, and fluorescence *in situ* hybridization (FISH) analysis.

Development of MDS is probably a multistep process with an initial genetic insult to the multipotent stem cells leading to the development of an abnormal clone [6–9]. The abnormal clone is the precursor of morphologically dysplastic and dysfunctional hematopoietic cells with a tendency to die prematurely. Excessive apoptosis (programmed cell death) of the hematopoietic precursors, particularly at the early stages, has been proposed as the primary mechanism for the bone marrow hypercellularity and peripheral cytopenia in patients with MDS [10–12]. According to some investigators, however, progression of the disease is accompanied by a decline in apoptosis

TABLE 8.1 WHO classification of MDS.*

Type	Blood findings	Bone marrow findings
Refractory anemia (RA)	Anemia, abnormal erythrocytes No or rare blasts	Dysplastic erythropoiesis <5% blasts <15% ringed sideroblasts
Refractory anemia with ringed sideroblasts (RARS)	Anemia, abnormal erythrocytes No blasts	Dysplastic erythropoiesis ≥15% ringed sideroblasts <5% blasts
Refractory cytopenia with multilineage dysplasia (RCMD)	Bicytopenia or pancytopenia No or rare blasts No Auer rods No absolute monocytosis	Multilineage dysplasia <5% blasts No Auer rods <15% ringed sideroblasts
Refractory cytopenia with multilineage dysplasia and ringed sideroblasts (RCMD-RS)	Bicytopenia or pancytopenia No or rare blasts No Auer rods No absolute monocytosis	Multilineage dysplasia <5% blasts No Auer rods ≥15% ringed sideroblasts
Refractory anemia with excess blasts-1 (RAEB-1)	Cytopenia(s) <5% blasts No Auer rods No absolute monocytosis	Unilineage or multilineage Dysplasia 5–9% blasts No Auer rods
Refractory anemia with excess blasts-2 (RAEB-2)	Cytopenia(s) 5–19% blasts Auer rods ± No absolute monocytosis	Unilineage or multilineage Dysplasia 10–19% blasts Auer rods ±
MDS associated with isolated del(5q)	Anemia Normal or increased platelets <5% blasts No Auer rods	Micromegakaryocytes Isolated del(5q) <5% blasts No Auer rods
Myelodysplastic syndrome, unclassifiable (MDS-U)	Cytopenia No or rare blasts No Auer rods	Unilineage myeloid Dysplasia <5% blasts No Auer rods

* Adapted from Ref. [1].

and increased levels of anti-apoptotic bcl-2 protein in the bone marrow progenitor cells in advanced MDS, such as RAEB. There are also reports of reversing the ratio of pro-apoptotic and anti-apoptotic proteins as the disease progresses. The possible contributing factors in the acceleration of apoptosis in the early developmental stages of MDS could be genetic damage and/or an altered marrow microenvironment.

Unlike bcl-2 which is an anti-apoptotic factor, the *C-MYC* gene is a pro-apoptotic regulator and seems to play a role in the pathogenesis of MDS at the early stages, such as RA and RARS [13–15].

There are several other genes that appear to be dysregulated in MDS (Figure 8.1). These include cell cycle regulatory genes, such as *EVI-1*, growth factors and angiogenesis genes, such as *TGF-α* and $-\beta$ and *VEGF*, receptor tyrosine kinase genes, such as *FLT3*, immunoregulatory cytokine genes, such as *IFN-γ* and *TNF-α*, and genes regulating

DNA methylation [16–18]. Abnormal DNA methylation of calcitonin and *p15* has been reported in MDS [19, 20].

The report of clinical autoimmune disorders in about 10% of MDS patients raises the possibility that bone marrow failure in MDS is immune mediated [21]. The autoimmune model for pathophysiology of MDS suggests an autoimmune T-cell attack to the bone marrow target cells causing an overproduction of pro-apoptotic cytokines, such as *IFN-γ* [22].

The ineffective hematopoiesis in MDS may involve one or several hematopoietic lines, resulting in anemia, thrombocytopenia, granulocytopenia, or pancytopenia. The lymphoid lineage may occasionally be involved. In such cases, evolution of acute leukemia at the later stages may be of a lymphoblastic type or biphenotypic [23, 24]. Lymphoid blast transformation is usually of B-cell type. The involvement of the lymphoid lineage in the myelodysplastic process may also cause immune dysfunction, such as cell-mediated

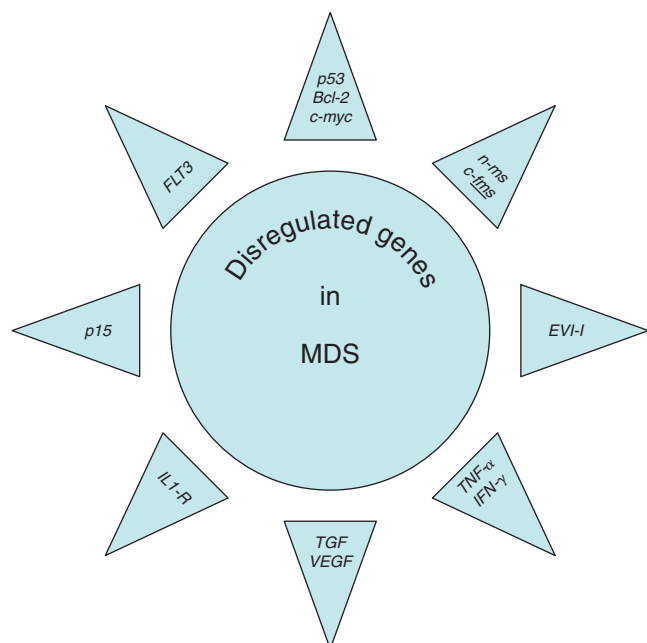


FIGURE 8.1 Numerous gene products appear to play important roles in pathogenesis of MDS.

suppression of bone marrow stem cells or deficient NK (natural killer) activities.

GENERAL MORPHOLOGIC FEATURES

The ineffective hematopoiesis in MDS is demonstrated by mono- or pancytopenia and abnormal morphology in one or more hematopoietic lines in bone marrow and peripheral blood [1, 25–27]. The bone marrow in patients with primary MDS is usually hyper- or normocellular, whereas patients with secondary MDS may show a variable marrow cellularity ranging from 5% to almost 100%. MDS bone marrow may show increased reticulin fibers with a higher frequency in the secondary MDS.

Bone marrow biopsy sections of MDS patients usually show some degree of topographical alterations, such as the presence of erythroid clusters next to the bone trabeculae, loss of sinusoidal orientation of megakaryocytes and their placement next to bone, and centrally located aggregates of myeloid precursors (Figure 8.2) [27, 28]. The morphologic appearance of aggregates of immature myeloid cells is referred to as “abnormal localization of immature precursors” (ALIP) (Figure 8.3). ALIP is defined as clusters of five or more myeloblasts and/or early immature myeloid cells, located in the marrow tissue away from bone trabeculae. More than three ALIP clusters per biopsy section are required to be diagnostically significant [1, 28]. Presence of ALIP, however, is not exclusive to MDS and has been observed in other hematologic conditions, such as myeloproliferative disorders and status post-bone marrow transplantation or chemotherapy [1, 28].

Signs of an inflammatory response, such as lymphoid aggregates, areas of edema, extravasation of erythrocytes, increased mast cells, plasma cells, and macrophages, disrupted sinusoids, and patchy or sometimes diffuse fibrosis are

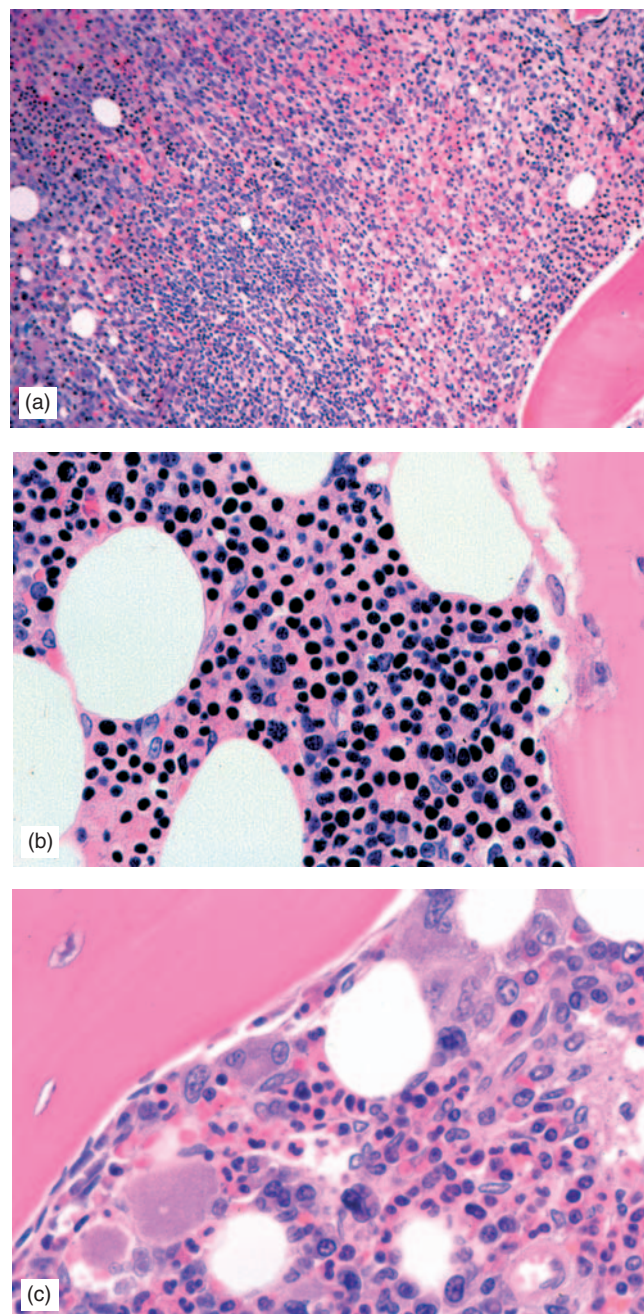


FIGURE 8.2 (a) Bone marrow section from a patient with MDS demonstrating hypercellularity with paratrabecular localization of the erythroid precursors and presence of a lymphoid aggregate. Higher power views demonstrate paratrabecular localization of erythroid precursors (b) and megakaryocytes (c).

frequent findings. Occasionally, there is evidence of hemophagocytosis and/or presence of sea blue histiocytes [25–27].

The accurate assessment of dysplastic cytologic features in blood and bone marrow smears depends on the quality of the smear preparations. Slides should be made from a fresh specimen and properly stained with preferably <2h of exposure to anticoagulants. The recommended threshold for significant dysplasia is at least 10% for each hematopoietic lineage [1]. The morphologic features of these dysplastic changes are discussed later.

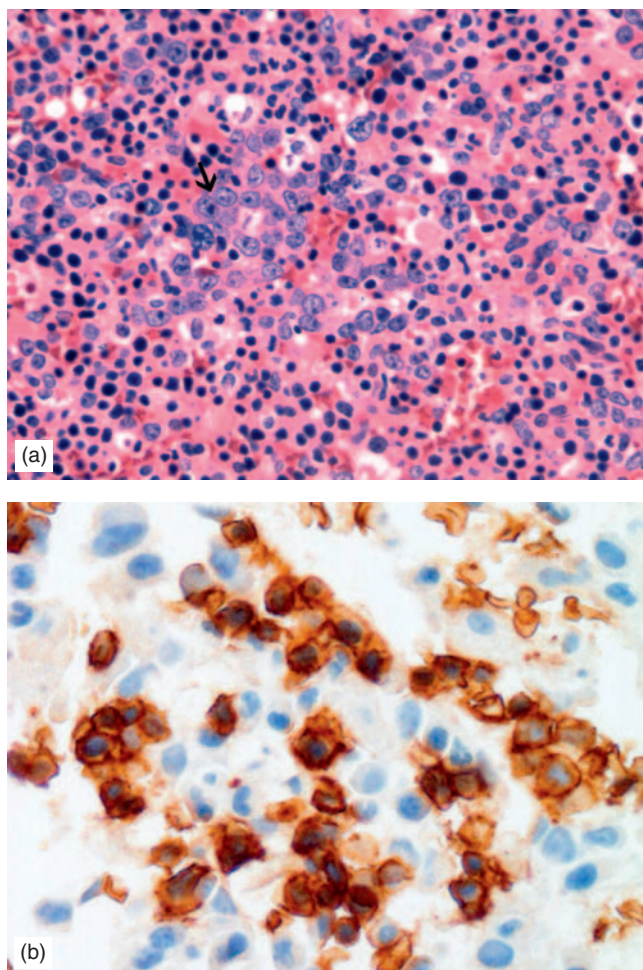


FIGURE 8.3 A hypercellular bone marrow biopsy section (a) showing several clusters of immature cells, referred to as “abnormal localization of immature precursors” (ALIP). The immunohistochemical stain (b) shows clusters of myeloperoxidase-positive immature cells.

Dyserythropoiesis

Dysplastic features of the erythroid precursors in bone marrow smears include megaloblastic changes, irregular nuclear shape, nuclear fragmentation and budding, multinucleation, nuclear bridging, cytoplasmic vacuolization, poor hemoglobinization, and presence of ringed sideroblasts and periodic acid-Schiff (PAS)-positive cytoplasmic globules (Figures 8.4 and 8.5). In biopsy sections, erythroid colonies may be seen next to the bone trabeculae.

Blood smears show a wide variety of abnormal erythrocyte morphology, such as macro-ovalocytosis, microcytosis, schistocytosis, basophilic stippling, and the presence of tear-drop-shaped red blood cells and Howell-Jolly bodies (Figure 8.6). Occasionally, nucleated red blood cells are present.

Dysgranulopoiesis

The granulocytic precursors may show abnormal variations in size, cytoplasmic granularity, and nuclear configuration (Figures 8.7–8.10) [1, 27, 29]. Abnormal staining of primary

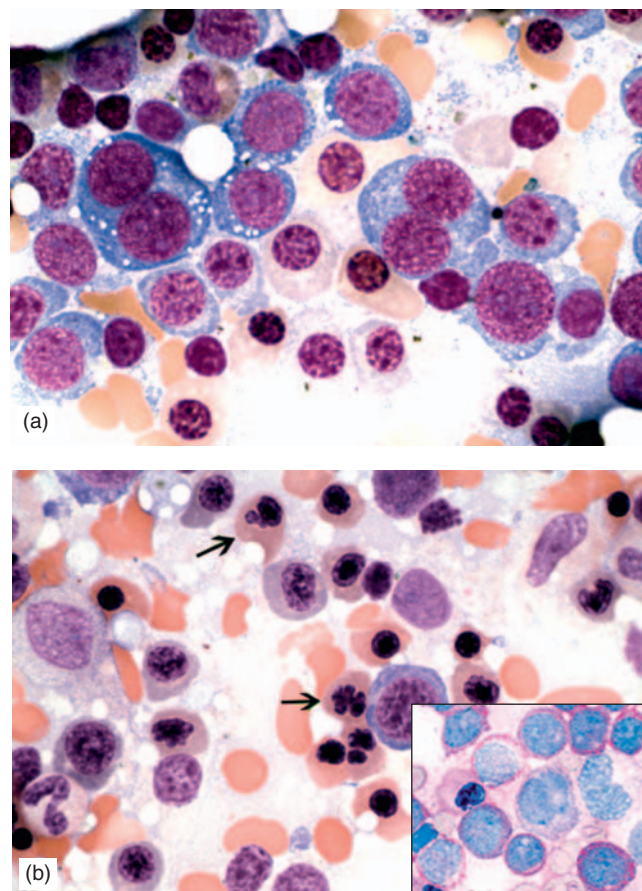


FIGURE 8.4 A bone marrow smear from a patient with RA showing dysplastic binucleated early erythroid precursors with vacuolated cytoplasm (a). PAS stain shows coarse PAS-positive granules in these cells (inset). Dysplastic late erythroid precursors with irregular or multilobated nuclei (arrows) are demonstrated in (b).

granules, hypergranularity or hypogranularity, is commonly observed in promyelocytes and myelocytes. Irregular distribution of the cytoplasmic basophilia may be present and the cytoplasm in the perinuclear area may stain lighter than that in the periphery. The more mature granulocytic cells may depict a marked variation in size and decreased or absent secondary granules. There may be coarse basophilic (pseudo-Chediak-Higashi) granules. Nuclear hyposegmentation (pseudo-Pelger-Huet anomaly) or hypersegmentation, and other forms of abnormal nuclear morphology, such as ringed (doughnut-shaped) nuclei, may be present. Eosinophils may be increased or show dysplastic changes, such as abnormal nuclear segmentation or abnormal granulation [30, 31]. Studies of bone marrow basophils on patients with MDS are very limited. However, basophilia has been observed in some MDS patients, and one report demonstrates lack of abnormal basophilic function in a group of patients with MDS [32, 33].

Abnormal Megakaryocytes and Platelets

Megakaryocytes may show multiple separated nuclei, hypo- or hyperlobated nuclei, vacuolated cytoplasm, and

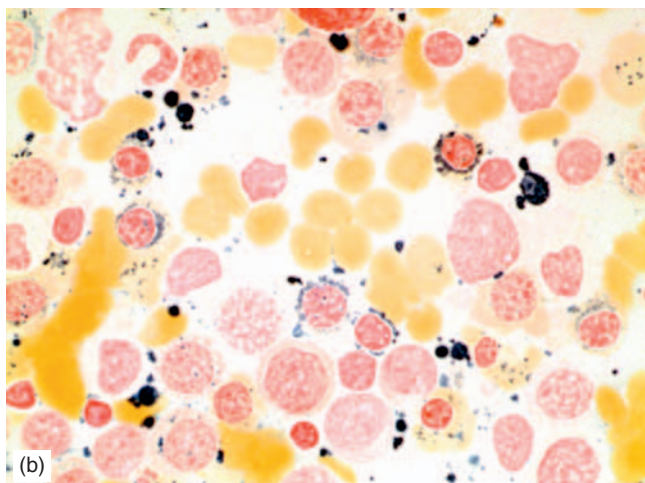
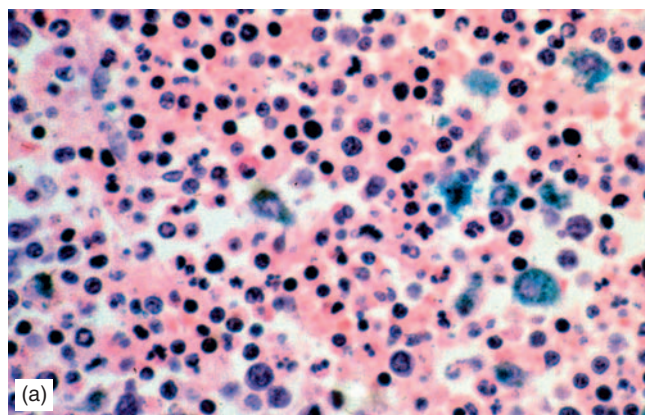


FIGURE 8.5 A hypercellular bone marrow biopsy section from a patient with RA showing erythroid preponderance with dysplastic changes (a). Numerous ringed sideroblasts are present in the bone marrow stained with Prussian blue (b).

giant abnormal cytoplasmic granules. Mono- and binuclear megakaryocytes are frequently seen (Figures 8.11 and 8.12) [1, 27, 29]. Sometimes, it is difficult to distinguish the mononuclear micromegakaryocytes from stromal cells or macrophages. In biopsy sections, megakaryocytes may appear in clusters or localized close to bone trabeculae. Immunohistochemical stains are helpful in distinguishing the dysplastic megakaryocytes from other bone marrow elements. Megakaryocytes express CD36, CD41, CD61, and factor VIII.

Blood smears show pleomorphic platelets with the presence of giant forms. They may show hypogranulation or abnormal granules [1, 29]. Megakaryocytic fragments, bare megakaryocytic nuclei, and sometimes micromegakaryocytes may be present [1, 29].

IMMUNOPHENOTYPIC STUDIES

Flow Cytometry

The dysplastic hematopoietic cells in MDS may show altered expression of the CD molecules. These changes may

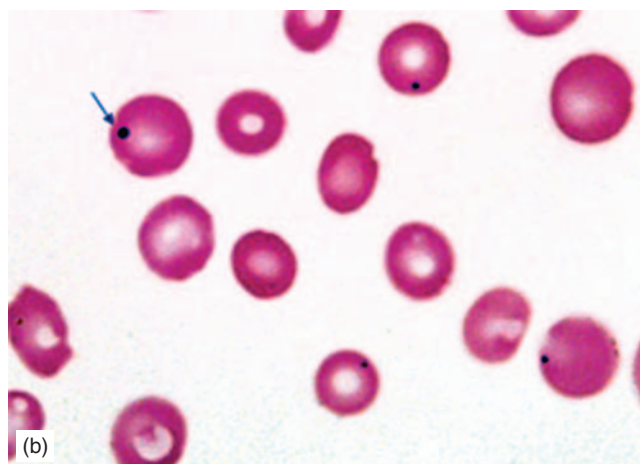
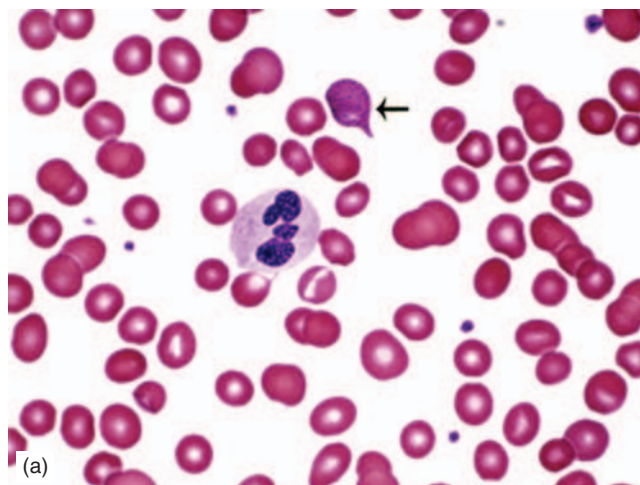


FIGURE 8.6 Patients with MDS often show abnormal erythrocyte morphology in peripheral blood smears, such as anisopoikilocytosis, teardrop (arrow), basophilic stippling (a), or Howell-Jolly bodies (b, arrow).

be detected by flow cytometry using a pattern-recognition approach and the measurement of the intensity, lack or aberrant expression of certain CD molecules. Although flow cytometry has not been utilized routinely for establishment of the diagnosis of MDS, numerous reports support its implementation as an accessory tool in evaluation of patients with a clinical history suggestive of MDS. These recommendations are based on the following difficulties in establishing the diagnosis [34, 35]:

1. A significant proportion of cases with MDS do not show increased myeloblasts or ringed sideroblasts.
2. Approximately half of the MDS patients may not show cytogenetic abnormalities.
3. Dysplastic changes are not pathognomonic of MDS and have been observed in a wide variety of non-clonal conditions, such as viral infections, post-chemotherapy, heavy metal toxicity, and folate or vitamin B12 deficiencies.

In addition, it has been shown that some of the phenotypic alterations may correlate with the cytogenetic

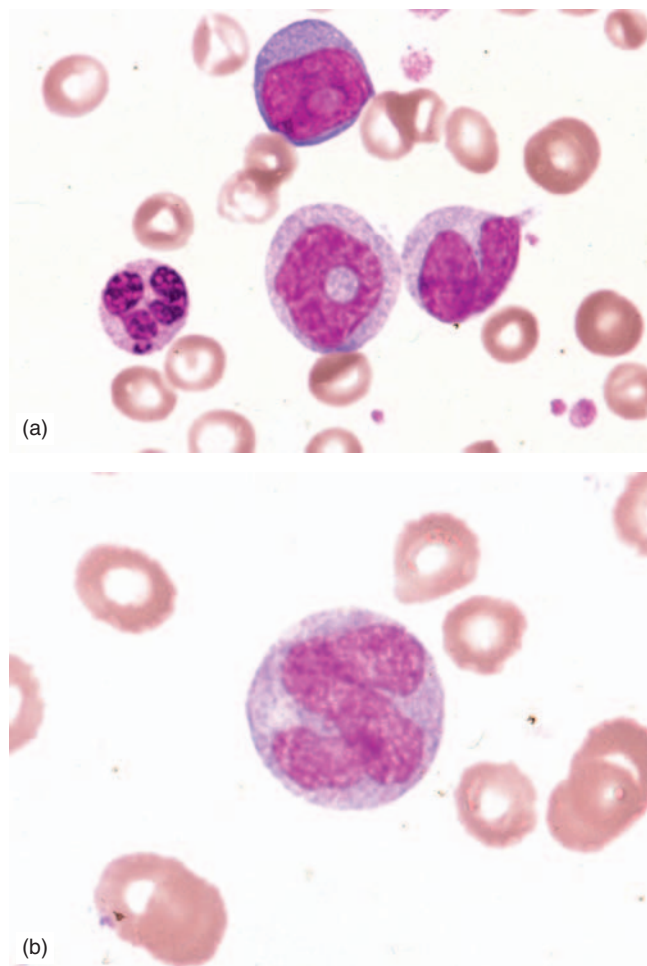


FIGURE 8.7 Blood smears showing a blast and an abnormal cell with doughnut-shaped nucleolus (a) and a dysplastic monocyte (b).

findings or clinical behavior of the disease. Therefore, flow cytometry is recommended as an accessory diagnostic workup in patients suspected for MDS.

The frequency of the hypogranularity of the myeloid cells in MDS often leads to a lower side scatter (SSC) in the flow cytometric dot plot analysis (Figure 8.13). There are also reports of increased expression of CD11a and CD66, reduced expression of CD10 and CD116, and aberrant expression of CD56 on the granulocytes in MDS [35, 36]. There may be an increased proportion of CD34-positive blast cells with frequent co-expression of CD4, CD11b, CD15, and/or CD56 (Figure 8.14) and over-expression of CD13, CD33, CD117, or CD133 [35, 36–38]. Also, a high HLA-DR and low CD11b on myeloblasts in MDS patients may be indicative of early conversion to acute leukemia [39]. There is a report of higher frequency of expression of CD7 and terminal deoxynucleotidyl transferase (TdT) (>60%) by the bone marrow blasts of MDS patients [40].

Erythroid precursors may show higher expression of CD105 and lower expression of CD71, as well as loss of A, B, and H antigens [36, 41]. Platelets may show decreased expression of CD41 and CD61. Several reports indicate that the number of hematogones (normal precursor B-cells), particularly type 1 (TdT⁺, CD34⁺, CD10⁺, CD19⁺),

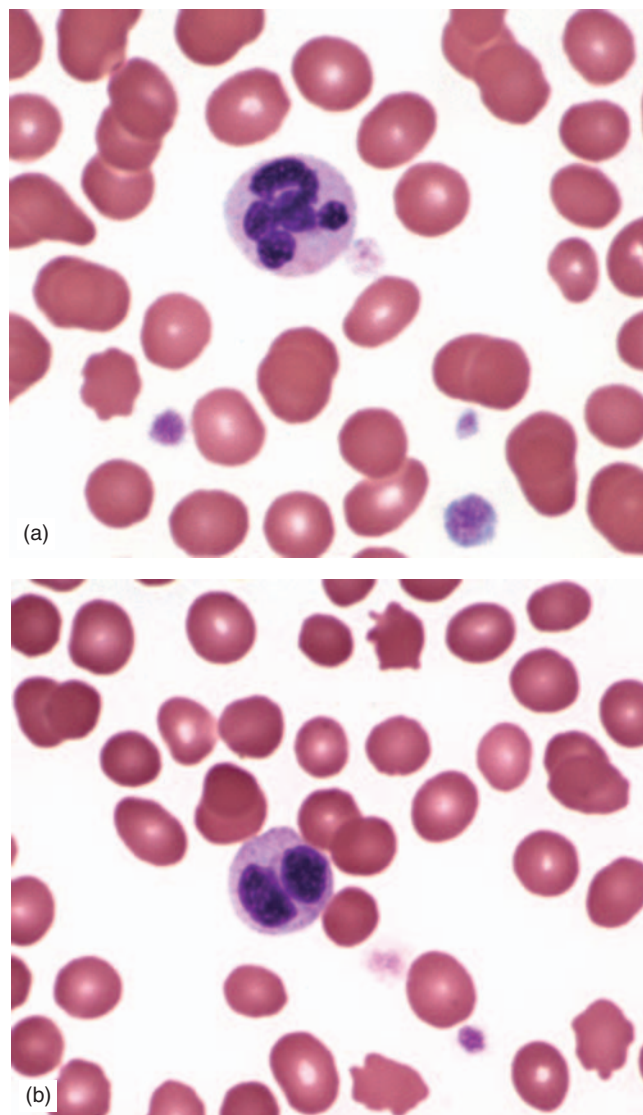


FIGURE 8.8 Blood smears showing a hypogranular and hypersegmented neutrophil (a) and a hyposegmented neutrophil (b).

is reduced in the bone marrow of MDS patient [37, 42]. A flow cytometric scoring system has been proposed for the diagnosis of MDS by Cherian and associates [43].

Immunohistochemical Studies

Immunohistochemical stains on bone marrow biopsies of patients suspected for MDS may provide helpful information regarding the following matters:

1. Evaluation of the topographical alterations and estimation of the M:E ratio by using monoclonal antibodies against hemoglobin and/or glycophorin A molecules for erythroid precursors and myeloperoxidase for myeloid precursors.
2. Detection of clusters of immature cells (ALIP) and estimation of blast cell numbers by using the blast-associated markers such as CD34 and CD117.

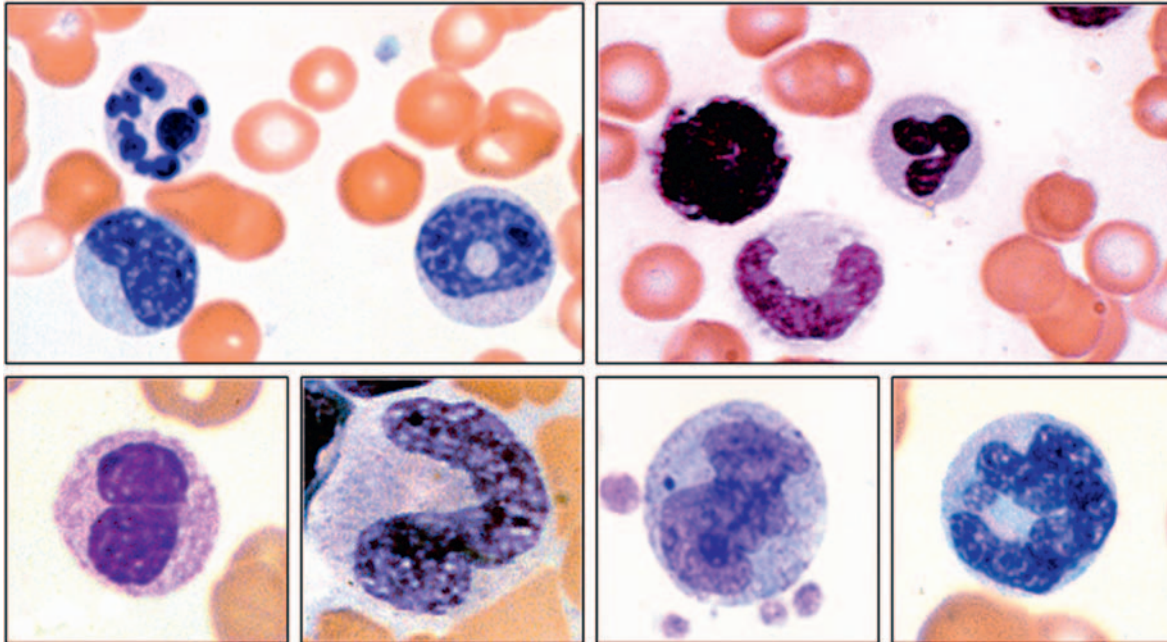


FIGURE 8.9 Blood smears from patients with MDS showing dysplastic neutrophils and monocytes.

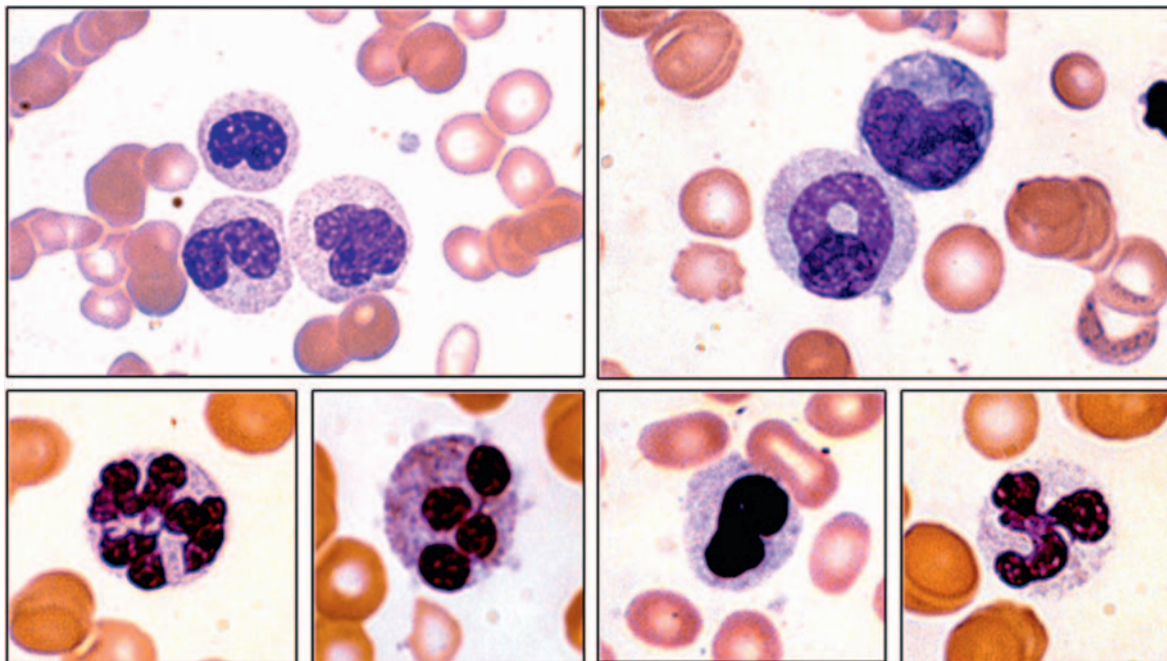


FIGURE 8.10 Blood smears from patients with MDS showing dysplastic neutrophils and monocytes.

3. Screening for the presence of micromegakaryocytes and topographical alterations of megakaryocytes by utilizing monoclonal antibodies against CD31, CD61, and factor VIII.
4. Evaluation of the monocytic component of the bone marrow by evaluating the results of CD68 and lysozyme stains.

Immunohistochemical stains are also occasionally used to evaluate the nature of the lymphoid aggregates, which are frequently observed in bone marrow biopsy sections of MDS patients. Sometimes these aggregates are morphologically atypical or are located next to the bone trabeculae, raising the possibility of a lymphoproliferative disorder.

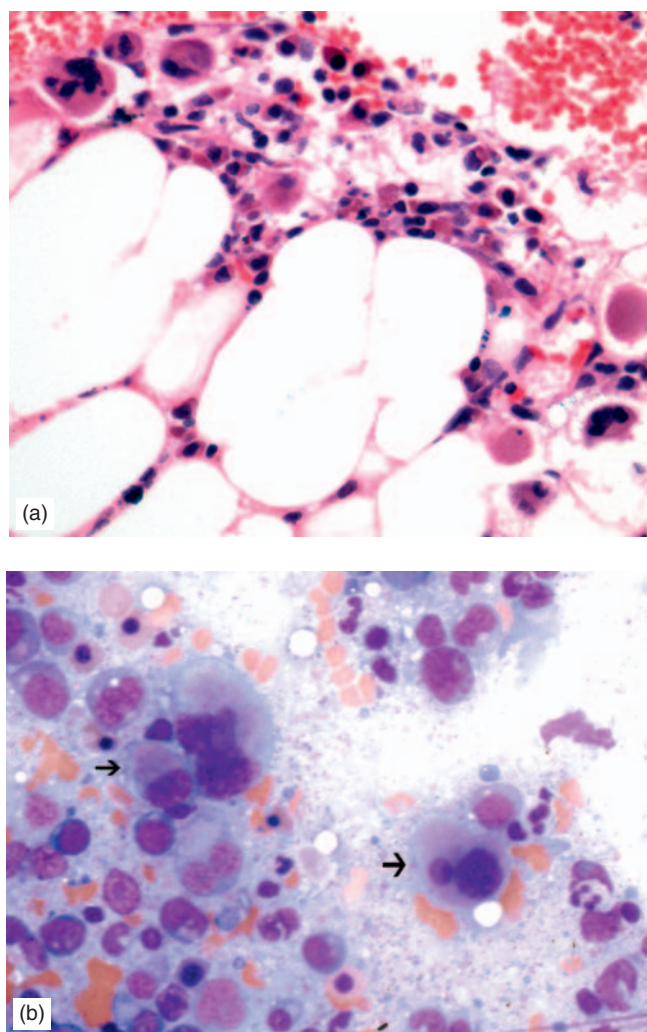


FIGURE 8.11 Clot section (a) and bone marrow smear (b) from a patient with 5q⁻ syndrome demonstrating numerous micromegakaryocytes.

MOLECULAR STUDIES

The diagnosis of MDS is typically based on clinical history and cell morphology in blood and bone marrow. Although clearly owing to one or more genetic defects in precursor stem cells, these are not yet sufficiently characterized to become targets for molecular diagnostic study. As such, the predominant molecular techniques used in MDS diagnosis are of the cytogenetic variety (FISH) to supplement the important standard karyotypic findings (see later). As already discussed, molecular techniques can be used to determine clonality of the underlying process, such as by characterization of polymorphic markers on the active and inactive X-chromosomes (applicable only in females). However, this is primarily a research tool and not directly relevant to the clinical diagnosis or management of a particular patient.

However, a number of individual point mutations, deletions, or epigenetic alterations have been observed in certain oncogenes, tumor suppressor genes, and signaling

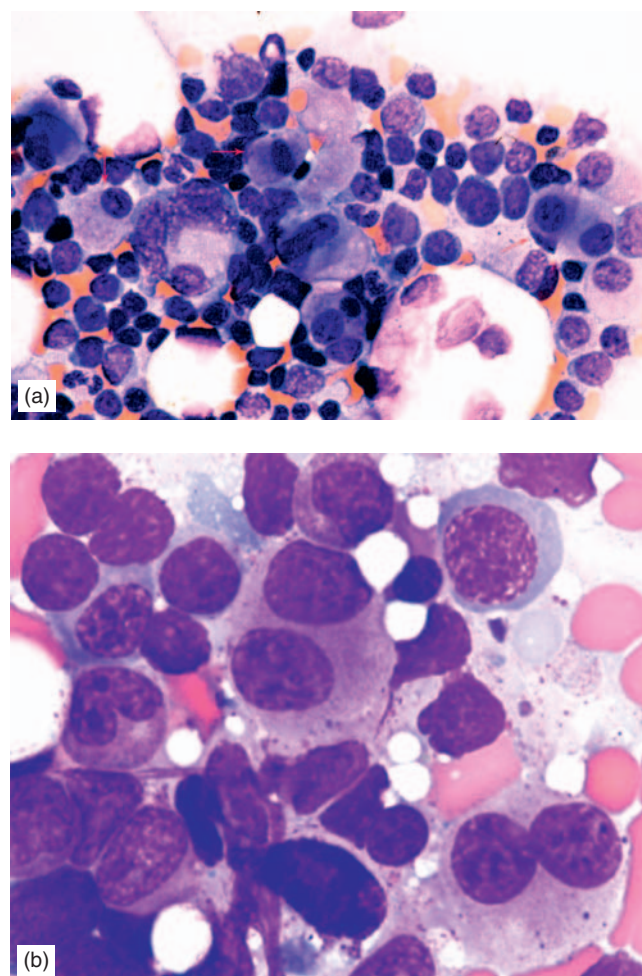


FIGURE 8.12 Micromegakaryocytes are frequent bone marrow findings in patients with MDS: (a and b) bone marrow smears.

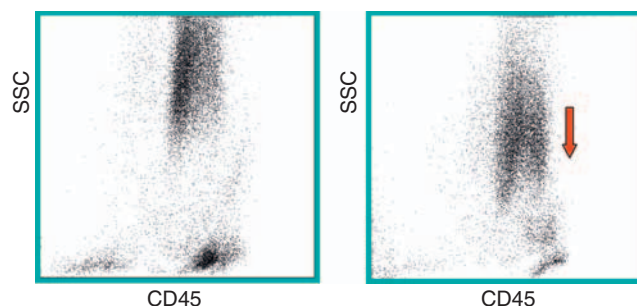


FIGURE 8.13 Dot plot analysis of side scatter (SSC) and CD45 of peripheral blood samples of a normal control (a) and a patient with MDS (b). There is a drop (arrow) in the SSC of the granulocytic population in the patient due to the hypogranularity of the granulocytes.

factors. The most frequent are point mutations in *N-ras* (found in 15–20% of MDS cases), tandem duplication mutation in the *FLT3* gene (5%), promoter methylation of *p15* (30–50%), inactivating and deletion mutations of *p53* (5–10%), and missense mutations in *AML1* [44].

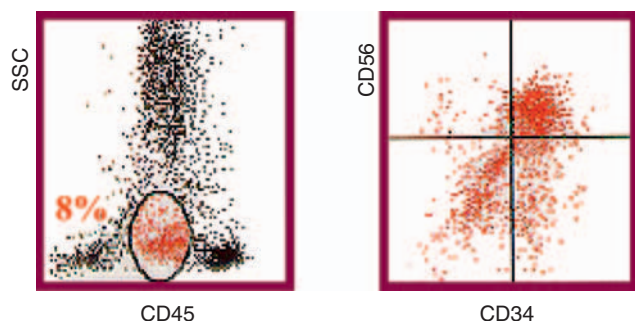


FIGURE 8.14 Flow cytometry of bone marrow from a patient with RAEB showing a population of blast cells co-expressing CD34 and CD56.

Inactivation of *p53* is of course a common occurrence in many types of cancer, but in MDS it appears to be an indicator of late stage or imminent progression to AML [45]. Again, although these findings may have important implications for our understanding of MDS pathogenesis and progression, they are not necessary or routinely used as targets for diagnosis.

CYTOGENETICS

Chromosomal changes occur in about 30–50% within the diverse subtypes of MDS and are the strongest independent prognostic indicators. These changes range from balanced translocations to unbalanced karyotypes with numerical or structural gains and losses as well as to complex aberrant karyotypes. The complex karyotypes are characterized by three or more chromosomal abnormalities and show an extremely unfavorable prognosis (Table 8.2) [46].

Conventional cytogenetics, FISH panel testing, and multiplex FISH (M-FISH) have analytical limitations. A combination of all three techniques should delineate the overwhelming majority of cytogenetic abnormalities in a bone marrow sample suspected for MDS.

Several recurrent and well-established cytogenetic changes have been described in MDS, and the detection of these changes can greatly facilitate diagnosis, prognosis, followup, and treatment of patients [47]. Although chromosomal abnormalities occur in almost half of *de novo* cases, aberrations are observed in up to 95% of secondary MDS. Most chromosomal defects in MDS are nonspecific, and with the exception of 5q–, none are specifically associated with any particular MDS subtypes [48].

Observed in nearly 50% of patients, chromosomal deletions are the most common defects in both *de novo* and secondary MDS. Deletions are generally interstitial, rather than terminal, and frequently occur in 5q, 7q, 20q, 11q, 13q, 12p, and 17p (Figures 8.15–8.19). Although a deletion observed as a sole abnormality is associated with low-risk MDS, deletions observed along with other abnormalities are associated with more advanced cases [48, 49].

Monosomies, trisomies, and unbalanced translocations are the next most common aberrations occurring

TABLE 8.2 Chromosomal abnormalities in MDS.

Type of abnormality	Chromosome	Prognosis
Numerical	–5	Good
	–7	Poor
	+8	Intermediate
	+13	
	+14	
	+15	
	+21	
Structural	der(1;7)(q10;p10)	
	t(3;21)(q26;q22)	
	ins(3;3)(q26;q21q26)	
	inv(3)(q21q26)	
	del(5)(q12-q31 or q31-35)	Good
	t(6;9)(p23;q34)	
	del(7)(q22)	Poor
	del(11q)	
	del(12)((p11p13)	
	del(13)(q12q14)	
	iso(17q)	Poor
	del(20)(q11q13) or	Poor
	del(20q)(q11.2)	
	idic(X)(q13)	
Normal karyotype		Good
Complex karyotype	3 or more abnormalities	Poor

in 15% of patients (Figures 8.20–8.23). The most common monosomies in MDS involve chromosomes 5, 7, and Y. Deletions and monosomies cause loss of one allele of a tumor suppressor gene with the subsequent submicroscopic deletion of the second allele on the homologous chromosome [43]. This recessive mechanism inactivates the cell's ability to control the cell cycle, DNA repair, and apoptosis [47, 48]. Although balanced translocations are relatively common aberrations in AMLs, they are very rare in MDS.

The most common of the chromosomal aberrations is represented by the chromosome 5q interstitial deletion. Deletion 5q occurs either as an isolated abnormality or accompanied by additional abnormalities, and accounts for up to 28% of all cytogenetic abnormalities in MDS with an overall frequency of approximately 15%. Prognosis of patients with primary MDS and isolated del(5q) is more favorable than that of those carrying an additional chromosomal abnormality. Patients with complex karyotypes have a poor prognosis with estimated survival of <6 months [50, 51].

The region of deletion in 5q chromosome is highly variable, but the most critically deleted region is about 1.5 Mb in size between bands 5q31 and 5q33. Only 5q33 deletions correspond to the 5q– syndrome and lead to a mild type of MDS, whereas 5q31 deletions are reported in other *de novo* and secondary subtypes and exhibit a more aggressive course.

The long arm of chromosome 5 has genes coding for many hematopoietic growth factors and growth factor



FIGURE 8.15 A G-banded female karyotype with a deletion of 5q.

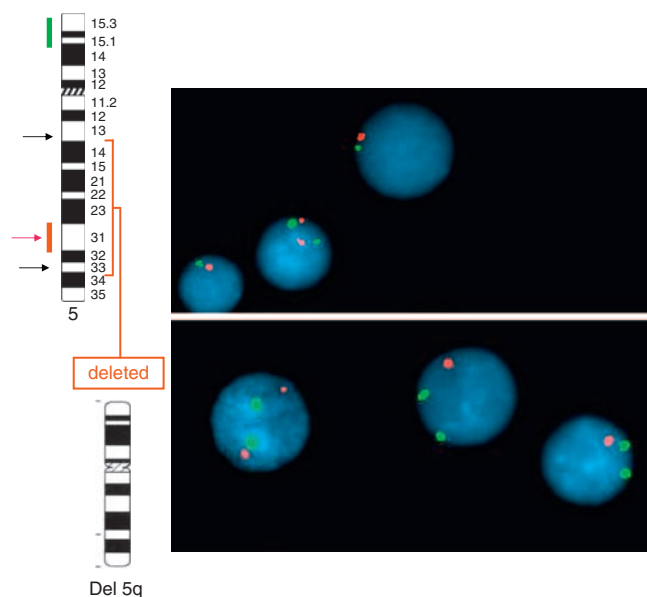


FIGURE 8.16 Ideogram of chromosome 5 showing the commonly deleted region in the long arm (red bracket). FISH studies on interphase cells show monosomy 5 (top right) and deleted 5q (bottom right).

receptors including IL-3, IL-4, IL-5, IL-9, and GM-CSF. Genome mapping studies of the commonly deleted region have identified about 40 genes with 33 of them being expressed by hematopoietic progenitor stem cells [52]. Some of these genes are also described to be tumor suppressor genes, transcriptional regulators, cytokines, and growth factors [53–55]. Although a tumor suppressor gene is postulated to reside in this region, a critical gene(s) related to leukemogenesis has not yet been definitively identified. Patients with the 5q deletion have distinct clinical and pathological features that include anemia, the

presence of dysplastic megakaryocytes in the bone marrow, and an indolent clinical course (see the following section).

Among patients who have MDS with a 5q deletion, the presence of one or more additional chromosomal abnormalities is associated with a more aggressive clinical course and considerably poorer overall survival as compared with patients who only demonstrate the isolated 5q deletion [49, 51].

7q⁻/-7 has been observed in all MDS subtypes, though it is much more common in advanced forms. 7q⁻/-7 occurs as a sole chromosomal abnormality in 1% of cases. 7q⁻/-7 is more common in secondary MDS, seen in up to 60% of the patients, and is therefore considered a secondary event in pathogenesis of the disease. Monosomy 7 is the most common chromosomal defect in bone marrow of patients with constitutional syndromes (e.g. Fanconi's anemia, type I neurofibromatosis, and severe congenital neutropenia) that predispose them to myeloid disorders [56]. Also, a recently described pediatric monosomy 7 syndrome presented with hepatosplenomegaly, leukocytosis, thrombocytopenia, male predominance, and an unfavorable outcome. Patients harboring deletions in the 7q31 to 7q36 regions have an inferior response to chemotherapy and shorter survival than those with deletions in the 7q22 region [57, 58].

Deletion of the long arm of chromosome 20 occurs in 5% of *de novo* and 7% of secondary MDS. This incidence might be an underestimation, since monosomy 20 and unbalanced translocations involving chromosome 20 occur as frequently as deletions. Although the critical region seems to be 20q11.2 to 20q12, deletions are rather large and involve most of the long arm of chromosome 20. Patients with del(20q) as a sole abnormality are in the low-risk MDS categories (RARS and RA), whereas those presenting with this deletion as a part of a complex karyotype (3 or more abnormalities) have a poor prognosis [59].

Deletion of the short arm of chromosome 17 encompasses not only simple deletions, but also unbalanced translocations, iso chromosome 17q, and (rarer) monosomies. Del(17q)



FIGURE 8.17 A G-banded karyotype showing a deletion of 7q, an ideogram of 7q with the two critical regions of deletion (black arrows), and a panel of interphase cells (bottom right) exhibiting 7q deletion by FISH.

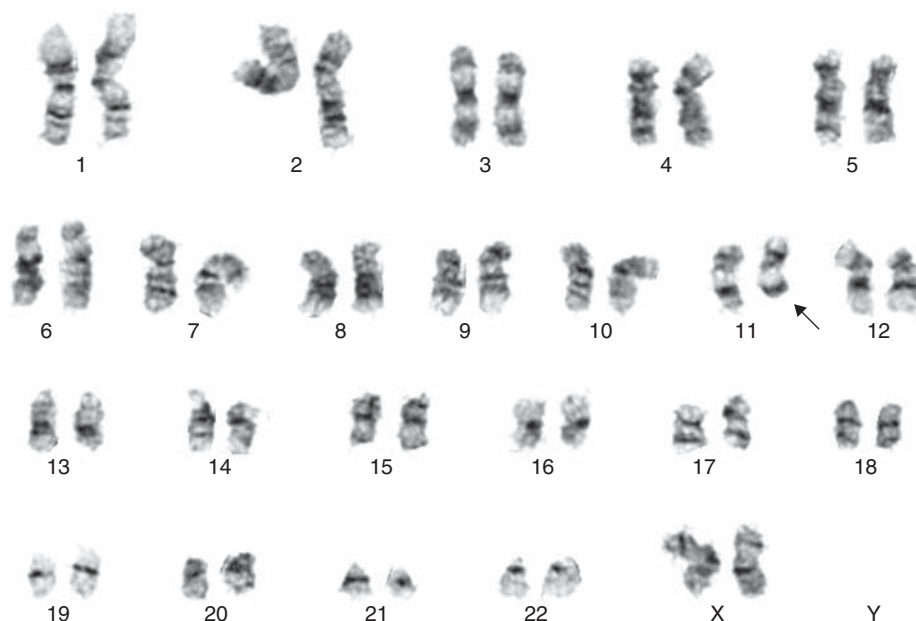


FIGURE 8.18 A G-banded karyotype with a deletion of 11q.

is rather rare in *de novo* MDS (~7%) but occurs more frequently in secondary MDS [60, 61]. Despite its heterogeneity, all the above-mentioned aberrations of the short arm of chromosome 17 lead to the loss of one p53 allele. Mutation or submicroscopic deletion of the other p53 allele occurs in 70% of the patients and cause inactivation of the gene.

Loss of the Y chromosome is observed in about 10% of MDS patients. It also occurs in about 7% of the elderly men without any hematological disorder. Therefore, MDS diagnosis cannot be based on the loss of chromosome-Y alone. When

biological and clinical parameters point to an MDS diagnosis, loss of the Y chromosome identifies patients with a favorable clinical outcome.

Interstitial deletions or balanced translocations involving band 12p13 are found in about 5% of patients with RAEB. These patients usually belong to an intermediate-risk cytogenetic category for MDS. However, recent studies suggest that 12p13 aberrations signify a clinical outcome similar to that of patients included within the low-risk category [59].

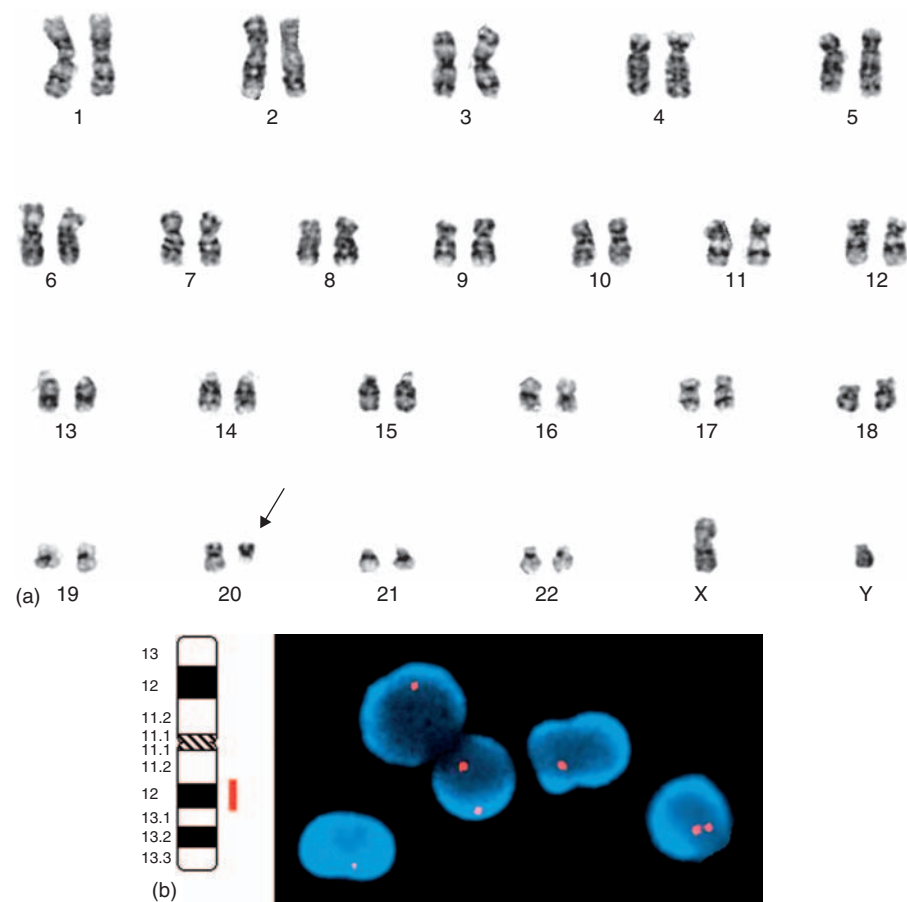


FIGURE 8.19 A G-banded karyotype showing a deletion of 20q (a) and the corresponding FISH studies (b).

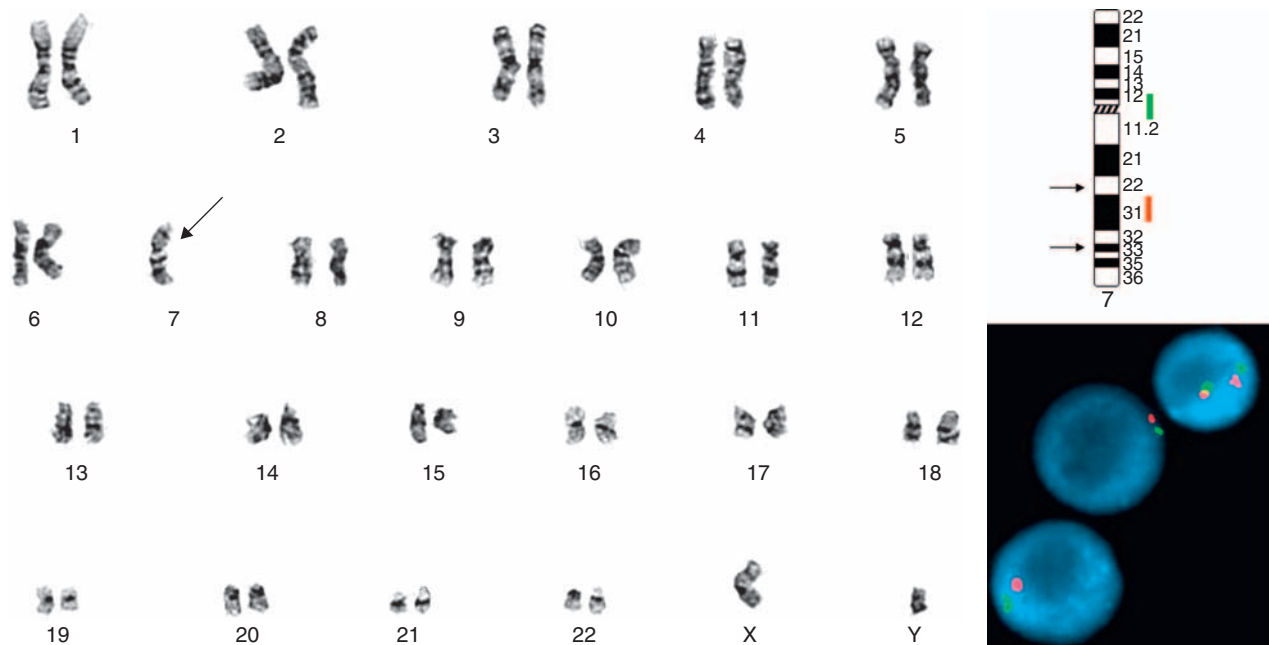


FIGURE 8.20 A G-banded karyotype with monosomy 7 and the corresponding FISH studies.

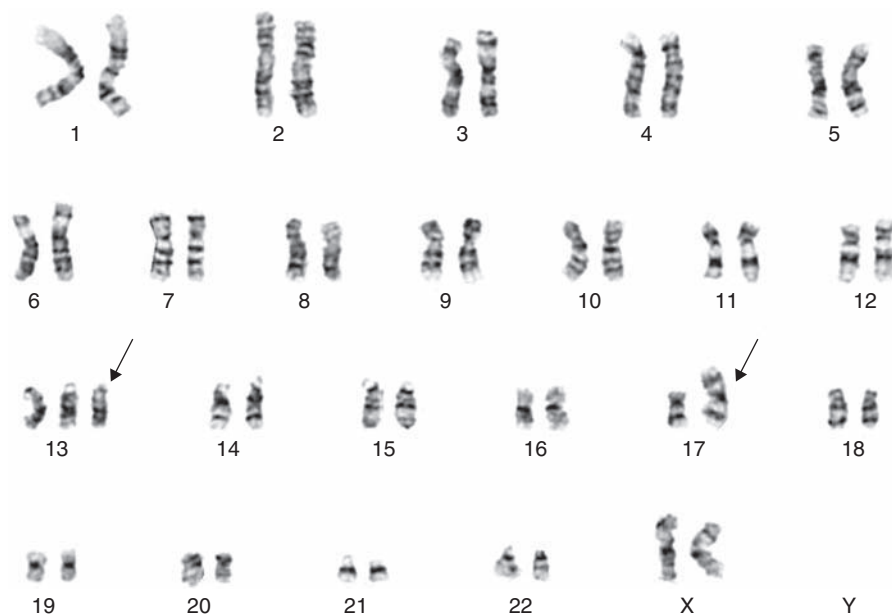


FIGURE 8.21 A G-banded karyotype showing trisomy 13 and deletion of 17p resulting from an isochromosome of 17q.

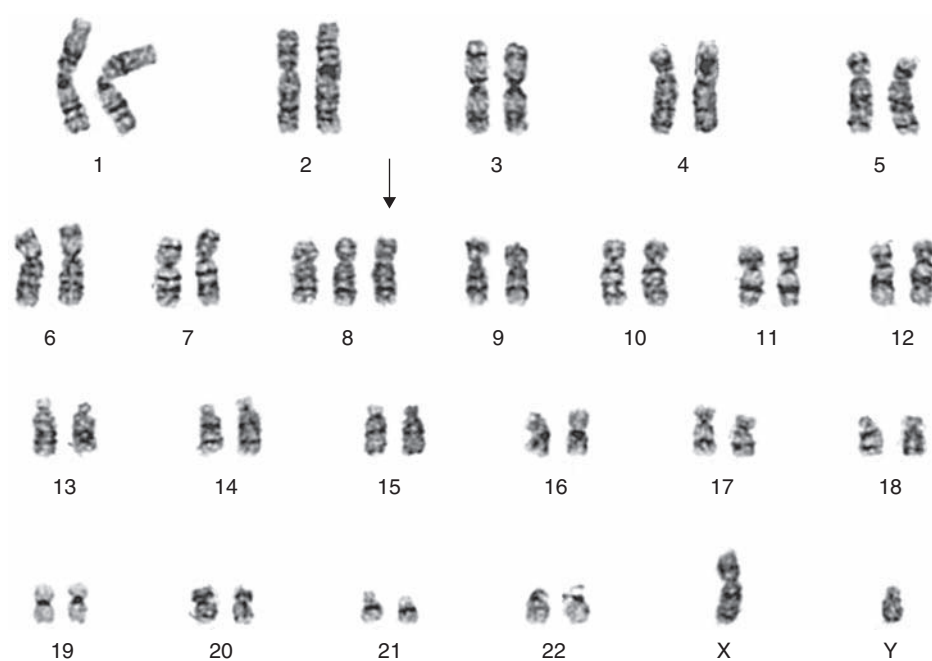
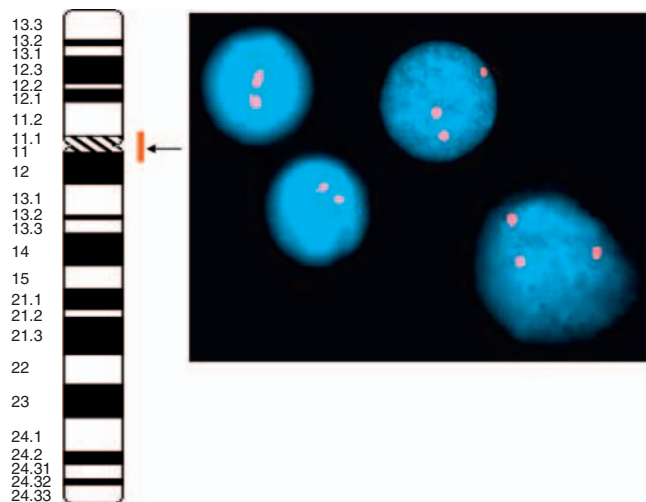


FIGURE 8.22 A G-banded karyotype with trisomy 8 and the corresponding FISH studies.



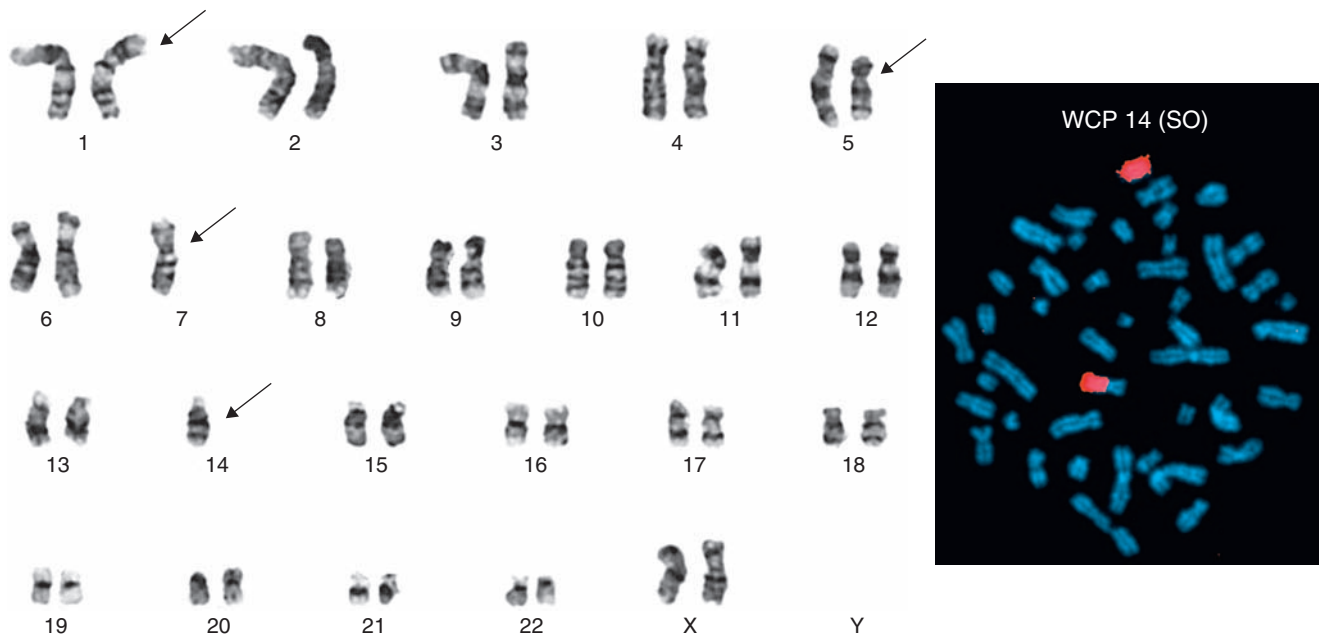


FIGURE 8.23 A G-banded karyotype showing a deletion of 1p, monosomy 7, and an unbalanced 5;14 translocation (left). The 5;14 translocation confirmed by whole chromosome paint of chromosome 14 (red signal) showing an unbalanced 5;14 translocation resulting in 5q deletion (right).

Several other chromosomal aberrations are observed in MDS, but are not specific to the disease. Trisomy 8 occurs in 10% of all MDS cases, but can be found in other clonal hematological disorders also. Trisomy 8 is more often associated with RARS and RAEB. Chromosome 3 rearrangements, typically translocations or inversions, occur in 2–5% of patients with MDS (also in AML). Chromosome 3 changes are frequently associated with $-7/7q$ and $5q-$, and are associated with short survival and a poor response to chemotherapy. Aberrations within 11q23 (the *MLL* gene locus) are found in 5% of MDS patients. Further karyotypic defects (e.g. rearrangements of the long arm of chromosome 3) occur in 5–10% of *de novo* MDS cases.

Based on cytogenetic abnormalities, MDS patients have been divided into three prognostic categories. Patients in the first, which is low-risk category, exhibit a normal karyotype, deletion of long arm of chromosome 5 as a sole abnormality, or harbor an isolated deletion of the long arm of chromosome 20. Patients with either a deletion of the short arm of chromosome 12 or trisomy 8 are categorized as an intermediate-risk group. Finally, the presence of complex karyotypes, monosomy 7, deletion of the short arm of chromosome 17, rearrangements involving chromosome 3, and aberrations of the long arm of chromosome 11 (*MLL*) indicates a high-risk group of MDS patients [49, 59, 62].

About 12% of patients with MDS with normal cytogenetics progress to AML, whereas 50% of those with chromosomal changes will progress to AML. Generally, therapy-related MDS (t-MDS) are much more clinically aggressive than primary MDS and this characteristic is reflected in the karyotypes. At least 80% of patients with t-MDS have chromosomally abnormal clones in the marrow and in a vast majority of cases these clones contain multiple abnormalities.

FISH and Other Technologies

It must be emphasized that no aberration is MDS-specific. However, if the aberration is one of those typically found in MDS, the diagnosis is strengthened. Lack of cytogenetic abnormality, however, is of no help in the diagnostic workup because one-third of patients with MDS have no chromosome aberrations. Also, there can be diagnostic problems due to difficulties in obtaining adequate bone marrow aspirate smears for evaluation of cytology. However, cytogenetic data can be obtained with FISH from nondividing or terminally differentiated cells, or from poor samples that contain too few cells for routine karyotyping studies. Detection of chromosomal abnormalities within interphase nuclei can be achieved by hybridizing with an appropriate selection of probes to the nuclei (Figures 8.16, 8.17, 8.19, 8.20, and 8.22). This technique permits the direct correlation of cytogenetic and cytologic features (e.g. trisomy 8 in a hypogranular neutrophil), which enables cytologists to differentiate malignant from benign conditions in equivocal cases [63].

One of the other advantages of FISH is that one can combine this method with cytology and/or immunohistochemistry to examine the cytogenetic pattern of specific cell populations to monitor the effects of therapy and to detect minimal residual disease. Most of the studies using FISH for the identification of lineage involvement in MDS indicate that, in most cases, the pluripotent stem cell is not affected because the lymphoid cells usually do not contain the chromosomal abnormality [64, 65]. Molecular cytogenetic techniques, such as M-FISH and spectral karyotyping (SKY), may allow for the comprehensive evaluation of the complex karyotypes. Although these techniques are used mostly in research, it is possible to analyze the origin of marker chromosomes, reveal cryptic rearrangements, and

determine recurrent breakpoints and the structure of derivative chromosomes.

CLASSIFICATION

According to the WHO classification, there are eight categories of MDS (Table 8.1) [1, 66–68]. These categories, based on the clinical course and survival rate, are divided into two major risk groups. The low-risk groups include RA with or without ringed sideroblasts and 5q– syndrome. RCMD and RAEB are considered high risk. A brief pathologic description of each MDS subclass is presented later, and the most frequent chromosomal aberrations are demonstrated in Table 8.3.

Refractory Anemia

Refractory anemia (RA) is a low-risk MDS with mono-lineage dysplasia characterized by anemia, dyserythropoiesis, and low percentage of blasts in bone marrow and peripheral blood (Figure 8.24). The degree of dysplasia in the erythroid precursors varies and may include megaloblastic changes, multinucleation, nuclear bridging, nuclear fragmentation or budding, cytoplasmic vacuolization, and abnormal hemoglobinization. Bone marrow is often hypercellular and frequently shows erythroid preponderance [1, 25, 29]. Myeloblasts account for <5% in the bone marrow and <1% in the peripheral blood. Ringed sideroblasts, if present, account for <15% of the erythroid precursors. The granulocytic and megakaryocytic lines are either normal or show minimal dysplastic changes. In the peripheral blood, red blood cells often show some degree of anisopoikilocytosis with reduced polychromasia (reticulocytes). In rare cases, instead of erythroid lineage, dysplasia is demonstrated in the megakaryocytic or granulocytic lineages.

According to a WHO recommendation, in order to establish the diagnosis of RA, all other etiological possibilities for erythroid abnormalities should be excluded [1]. These possibilities include drug and chemical exposure,

immunologic disorders, viral infections, congenital abnormalities, and vitamin deficiencies. Also, there should be an observation period of at least 6 months if cytogenetic and molecular studies show no evidence of clonal disorder. Development of multilineage dysplasia and/or an increase in the percent of blast cells are suggestive of progression of the disease into a more aggressive type of MDS [1, 66, 68].

Refractory anemia represents about 5–10% of the MDS cases; it usually affects elderly people and has no known etiology so far.

Refractory Anemia with Ringed Sideroblasts

Refractory anemia with ringed sideroblasts (RARS) is a low-risk MDS characterized by anemia, dyserythropoiesis, and presence of 15% or more ringed sideroblasts in the erythroid precursors [1, 66, 68]. Myeloblasts are not present in peripheral blood and account for <5% of the total cells in the bone marrow. Similar to RA, the degree of erythroid dysplasia in RARS varies and may include megaloblastic changes, multinucleation, nuclear bridging, fragmentation or budding, cytoplasmic vacuolization, and abnormal hemoglobinization [1, 25, 29]. The granulocytic and megakaryocytic lines are either normal or show minimal dysplastic changes. Bone marrow is hypercellular and shows erythroid preponderance with the presence of 15% or more ringed sideroblasts in erythroid precursors (Figure 8.25). Ringed sideroblasts represent nucleated red cells with precipitated iron particles in their mitochondria. Since mitochondria are often located around the nucleus, iron stain shows positively stained (siderotic) granules surrounding the nucleus like a ring. Ringed sideroblast is defined as an iron-containing erythroid precursor (sideroblast) with 10 or more siderotic granules encircling one-third or more of the nuclear parameter [1]. The bone marrow iron stain often shows increased iron stores with numerous hemosiderin-laden macrophages.

In the peripheral blood, red blood cells often show some degree of anisopoikilocytosis with reduced polychromasia (reticulocytes). A dimorphic morphologic pattern is common with a mixture of normochromic and hypochromic red cells. There is no absolute monocytosis (monocyte count $<1 \times 10^9/L$) [1, 25, 29].

Approximately 10% of the MDS cases are of RARS type. RARS is primarily a disease of old age, more frequently occurring in men than in women. Etiology of RARS is not known.

Refractory Cytopenia with Multilineage Dysplasia

Refractory cytopenia with multilineage dysplasia (RCMD) is characterized by the presence of dysplasia in two or more hematopoietic lineages and corresponding cytopenias [1]. The spectrum of dysplastic changes is discussed earlier in the section “General Morphologic Features.” At least 10% of the precursor cells in two or more hematopoietic lineages show dysplastic changes (Figure 8.26) [1]. Bone marrow is usually hypercellular and contains <5% myeloblasts. Occasional blasts

TABLE 8.3 Incidence of chromosomal aberrations in MDS subclasses.

Subgroup	Common alterations	Frequency (%)
RA	del(5q)	50
	–7	10–15
	–8	20
RARS	+8	30
	del(5q)	25
	del(11q)	10
	del(20q)	10–15
RAEB	–5/del(5q)	35–40
	–7/del(7q)	30–35
	+8	20

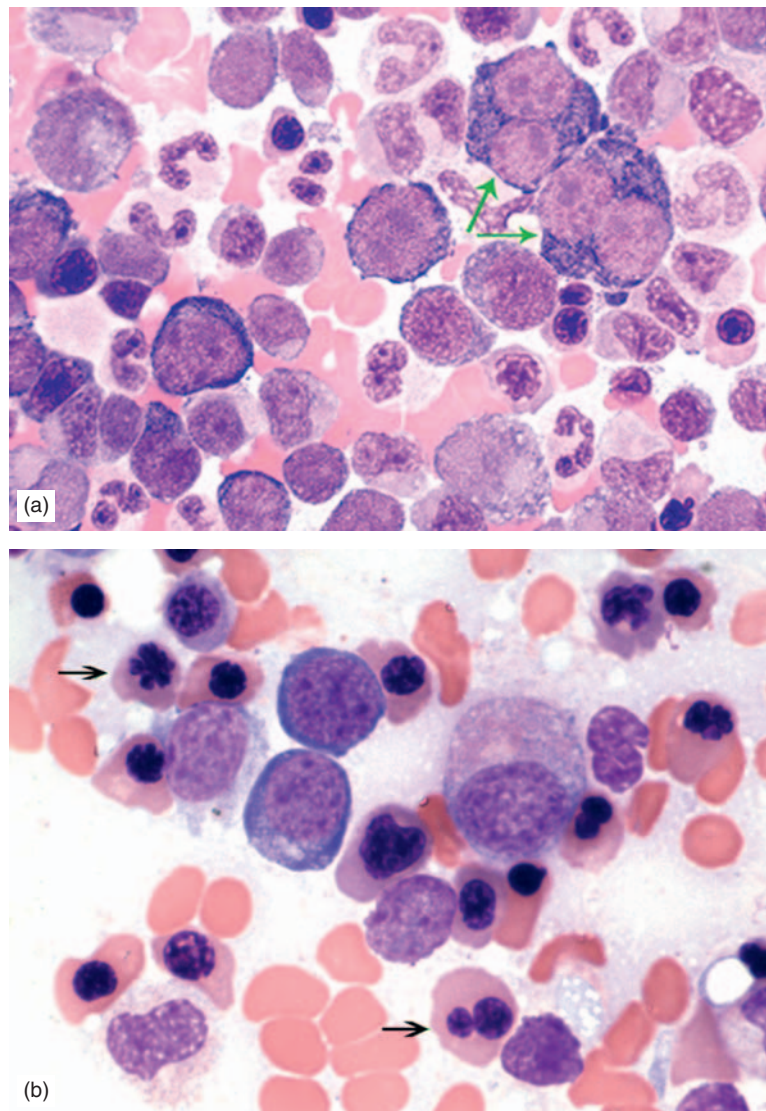


FIGURE 8.24 Refractory anemia: bone marrow smears demonstrating early dysplastic binucleated erythroid forms (a, arrows) and dysplastic late erythroid precursors with irregular or lobated nuclei (b, arrows).

may be present in the peripheral blood. There is no absolute monocytosis (monocyte count $<1 \times 10^9/L$).

Iron stores are often increased and ringed sideroblasts may be present, but they account for $<15\%$ of the erythroid precursors. The condition is referred to as RCMD-RS when ringed sideroblasts are 15% or more [1].

RCMD and RCMD-RS account for about 15–24% and 10–15% of MDS cases, respectively. They usually affect elderly people and have no known etiology so far.

Refractory Anemia with Excess Blasts

Refractory anemia with excess blasts (RAEB) is characterized by multilineage dysplasia and increased myeloblasts (5–19%) in the bone marrow and/or peripheral blood (1–19%) [1, 66, 69]. The spectrum of dysplastic changes is discussed earlier in the section “General Morphologic Features.” At least 10%

of the precursor cells in two or more hematopoietic lineages show dysplastic changes. Bone marrow is usually hypercellular and shows myeloid preponderance and left shift with increased blasts accounting for 5–19% of the bone marrow cells (Figures 8.27 and 8.28) [1, 25, 29]. The centrally located small aggregates of blasts, referred to as ALIP, are often present in the biopsy sections [1, 29]. The bone marrow biopsy in $<15\%$ of the cases is hypocellular. Peripheral blood smears show multilineage dysplasia, myeloid left shift, and the presence of 1–19% myeloblasts. There is no absolute monocytosis (monocyte count $<1 \times 10^9/L$).

Refractory anemia with excess blasts is divided into two groups: RAEB-1 and RAEB-2 [1].

RAEB-1 refers to the group with 5–9% blasts in the bone marrow and/or $<5\%$ blasts in the peripheral blood. The disorder is called RAEB-2 if the bone marrow blasts are 10–19% of the total cells and/or there are 5–19% blasts in the peripheral blood.

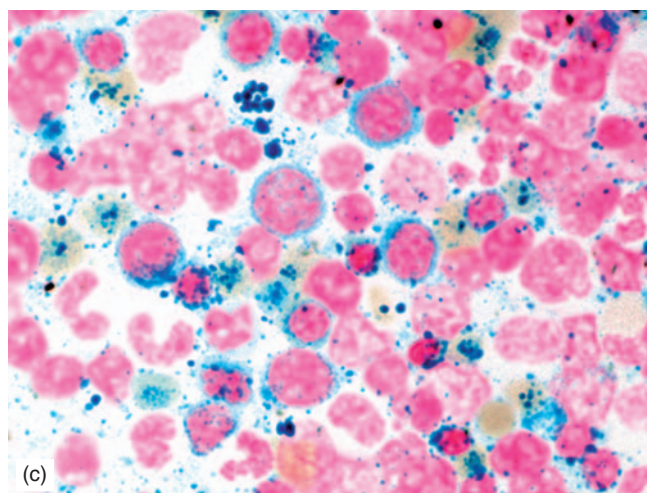
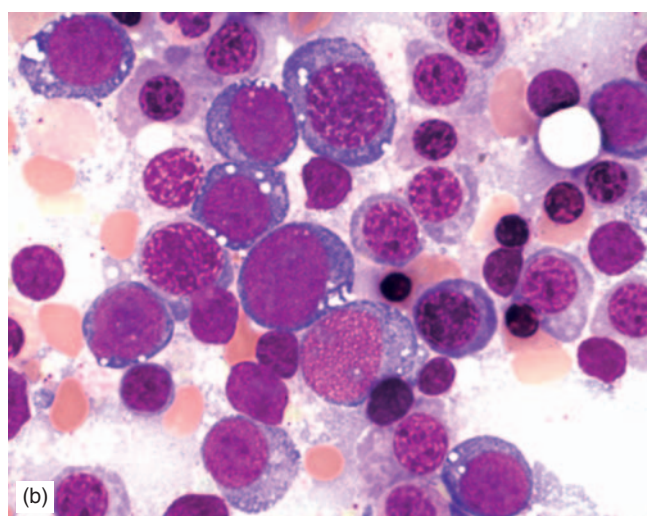
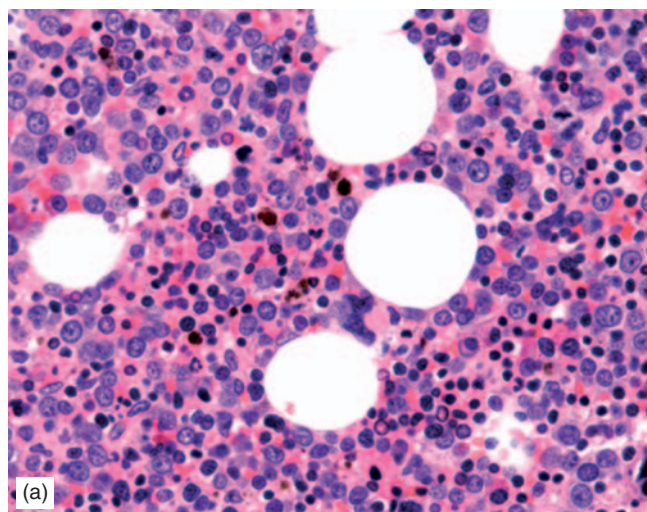


FIGURE 8.25 Refractory anemia with ringed sideroblasts: bone marrow biopsy reveals hypercellularity and erythroid preponderance (a); bone marrow smear shows erythroid preponderance and dysplastic vacuolated early erythroid precursors (b); and numerous ringed sideroblasts are present by iron stain (c).

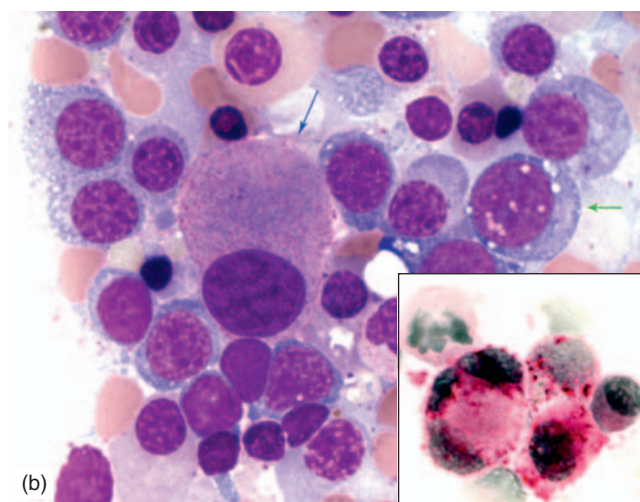
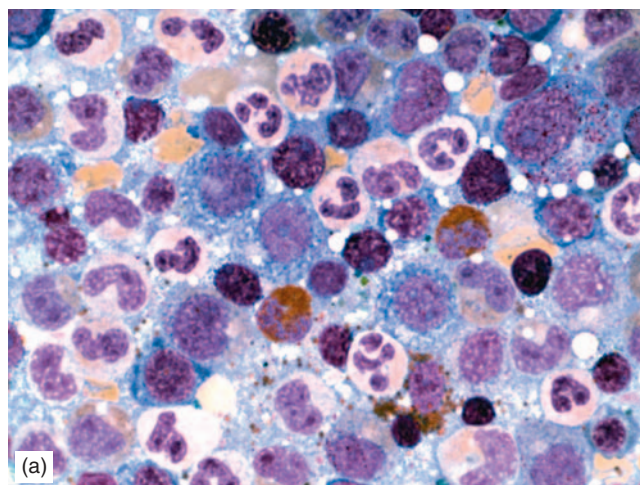


FIGURE 8.26 Bone marrow smears from a patient with refractory cytopenia with multilineage dysplasia demonstrating erythroid dysplasia (green arrow) and hypogranular neutrophils (a) and a micromegakaryocyte (b, blue arrow). The inset demonstrates coarse PAS-positive granules in a micromegakaryocyte and an erythroid precursor.

Refractory anemia with excess blasts accounts for about 30–40% of the MDS cases, and it usually affects patients older than 50 years. Etiology of RAEB is not known.

The 5q– Syndrome

5q– syndrome [MDS associated with isolated del(5q31-33) chromosome abnormality] is characterized by RA, which is usually macrocytic, normal to elevated platelet counts, modest leukopenia, and the presence of numerous micromegakaryocytes in the bone marrow (Figures 8.15 and 8.29) [1, 70, 71]. Bone marrow biopsies are usually hypercellular and show myeloid preponderance. Bone marrow smears show small megakaryocytes with mono- or hypolobated nuclei and <5% blasts [1, 25, 29]. Erythroid precursors often show dysplastic changes. Blood smears show macrocytosis, mild leukopenia, and, sometimes, occasional blasts.

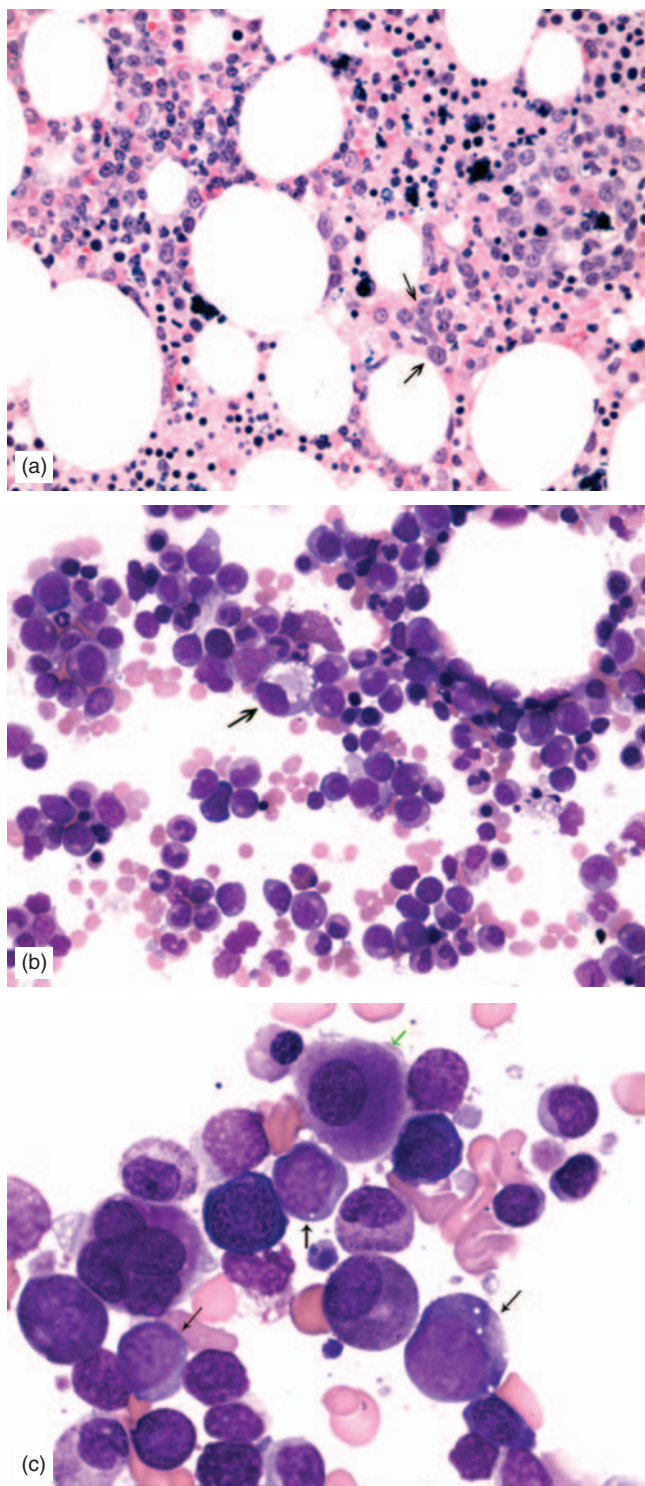


FIGURE 8.27 Bone marrow biopsy section is left shifted with increased number of immature cells (a, arrows). Bone marrow smears showing increased blasts (black arrows) and micromegakaryocytes (green arrows): (b) low power and (c) high power.

The etiology of this syndrome is not known, but the deleted region on the long arm of chromosome 5 (1.5 Mb at 5q31-q32) is the home of a number of important genes, such as IL-9 and EGR-1 [70, 71]. However, so far, a tumor

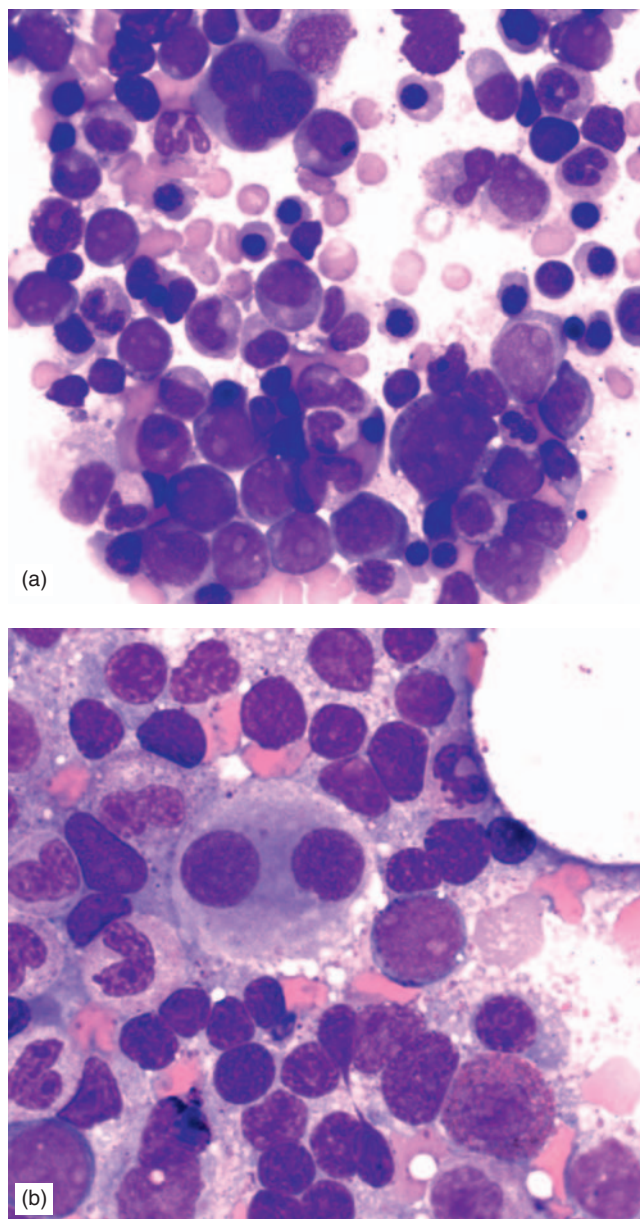


FIGURE 8.28 Refractory anemia with excess blasts: Bone marrow smears show increased proportion of myeloblasts and a trinucleated micromegakaryocyte (a) and a binucleated micromegakaryocyte (b).

suppressor gene responsible for MDS has not been identified on this chromosomal region.

5q- syndrome is considered a subtype of low-risk MDS, which predominantly affects elderly women. It accounts for about 10% of the MDS cases [70].

MDS, Unclassifiable

This term is recommended by WHO for those MDS cases that lack proper features to fall into one of the well-defined, above-mentioned categories, such as RA, RARS, RCMD, and RAEB [1]. Patients in this category usually show dysplastic changes in the granulocytic and/or megakaryocytic

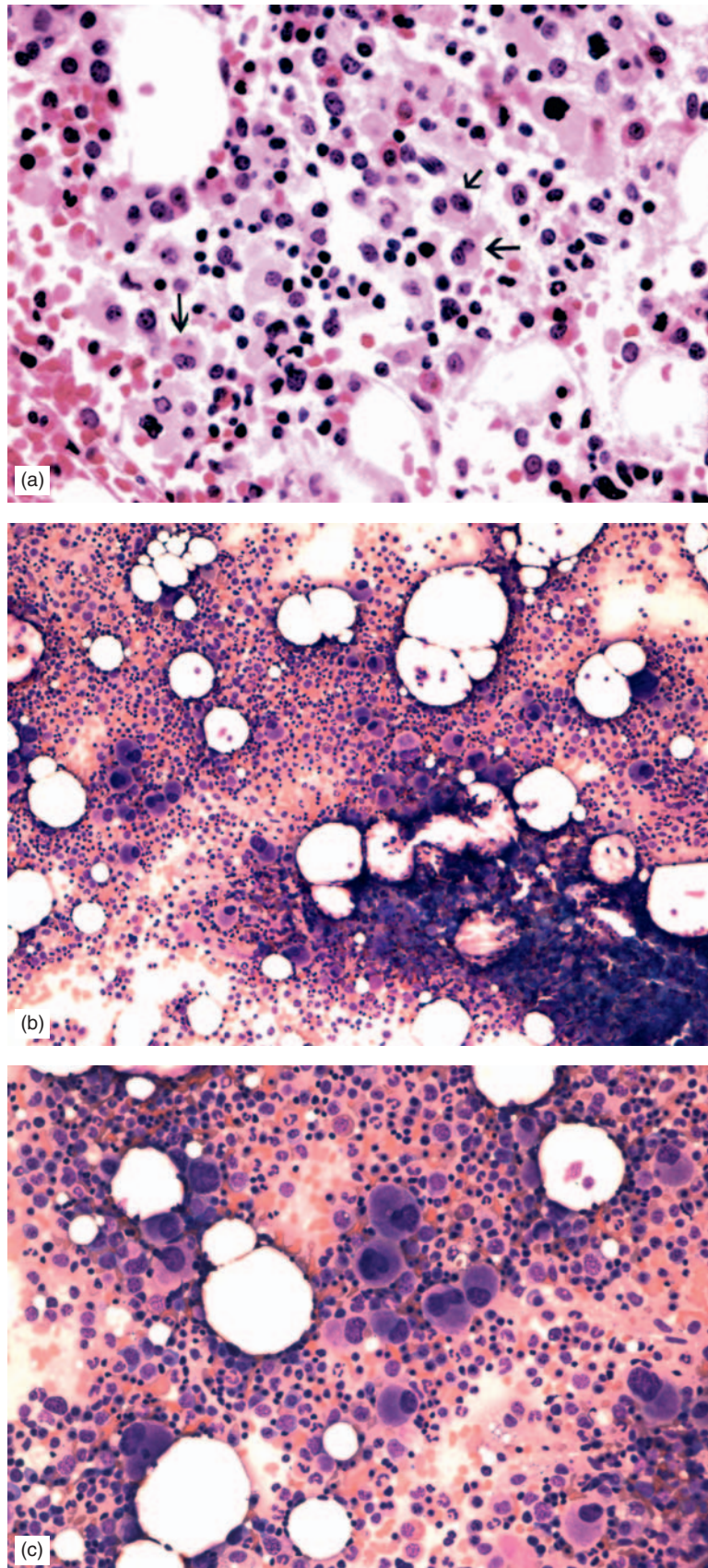


FIGURE 8.29 5q[−] syndrome is characterized by the presence of numerous micromegakaryocytes in the bone marrow: (a) biopsy section and (b) bone marrow smear.

lineages along with granulocytopenia and/or thrombocytopenia. Bone marrow is frequently hypercellular, but it could be normo- or hypocellular. There is no evidence of increased blasts.

OTHER TYPES OF MDS

Therapy-Related (Secondary) MDS

The development of MDS following cytotoxic chemotherapy and/or radiotherapy has been extensively investigated and reported in the literature [72–75]. The emergence of t-MDS is usually associated with a long latency period and is particularly seen following the use of alkylating agents. The pathologic manifestations of t-MDS are, in general, similar to those of primary MDS except for higher frequency of the following features [1, 72–75]:

- 1. High-risk variants
- 2. Bone marrow fibrosis
- 3. Bone marrow hypocellularity
- 4. Unclassifiable forms
- 5. Chromosomal aberrations (Table 8.4)
- 6. Transformation to acute leukemia.

t-MDS represents one spectrum of a broader syndrome now designated as *therapy-related AML and myelodysplasia* (see Chapter 12). It constitutes about 10–15% of the total MDS cases. The interval between initiation of therapy and the onset of MDS varies and depends on the type, duration, and dose of the therapeutic agent(s). This period in most studies ranges from 1 to 8 years, with a mean of 5 years [72–75]. The topoisomerase II inhibitors may occasionally cause MDS but more often are associated with *de novo* acute myelogenous leukemia without going through dysmyelopoiesis. Prolonged environmental or occupational exposure to benzene and benzene-derivative compounds may also lead to MDS.

Pediatric MDS

Myelodysplastic syndromes are rare in children, accounting for approximately 3–5% of all pediatric clonal hematologic disorders [76, 77]. They may also present themselves differently from the adult forms, particularly the MDS categories with <5% blasts. The following observations have been reported in pediatric patients with MDS [76–81]:

- 1. Hematopoietic dysplasia is frequently observed in a variety of conditions, such as infection, metabolic disorders, and nutritional deficiencies.
- 2. Neutropenia and thrombocytopenia are more frequently observed than anemia.
- 3. The rate of cytogenetic aberrations in low-risk MDS is higher in children (about 65%) than in adults

TABLE 8.4 Comparison of chromosomal aberrations between primary and secondary MDS.

Abnormality	Primary MDS (%)	Secondary MDS (%)
del(5q)	10–20	20
Monosomy 7	10–15	30–50
Trisomy 8	15	10
Loss of 17p	3	10

(20–30%). Monosomy 7 is one of the most frequent findings.

- 4. The majority of the cases fall into the MDS/myeloproliferative categories (see Chapter 10), particularly in patients younger than 5 years.

Hypocellular MDS

Hypocellular MDS accounts for about 5–10% of the MDS cases [82, 83]. This variant is usually therapy related and is often associated with more severe pancytopenia. Most investigators consider the diagnosis of hypocellular MDS when bone marrow cellularity is ≤25% of the age-matched normal range (Figure 8.30). Aplastic bone marrow conditions (such as aplastic anemia; Fanconi anemia, FA; and paroxysmal nocturnal hemoglobinuria, PNH) and hypocellular variants of hairy cell and acute leukemias are in the list of differential diagnosis [84]. Dysplastic hematopoiesis distinguishes hypocellular MDS from the bone marrow aplasia group, and blast counts of <20% separate this entity from acute leukemias. Dysplastic erythroid and myeloid cells are present in the blood smears and/or the bone marrow samples, and abnormal megakaryocytes are often identified. Estimation of blast number is facilitated by immunophenotypic studies by using blast-associated markers, such as CD34 and CD117.

Non-clonal Myelodysplasia

Non-clonal myelodysplastic changes have been observed in a variety of conditions, such as autoimmunity, infections, nutritional deficiencies, heavy metal intoxication, and post-chemotherapy and/or radiotherapy. Dysplastic changes in these conditions are often reversible upon elimination of the causative factors, and are not associated with chromosomal aberrations. Representative examples of non-clonal MDS are briefly discussed in the following sections.

Autoimmune Myelodysplasia

Myelodysplasia has been observed in a small proportion of patients with autoimmune disorders [21]. The dysplastic changes in these patients are not associated with chromosomal aberrations, and patients respond positively to

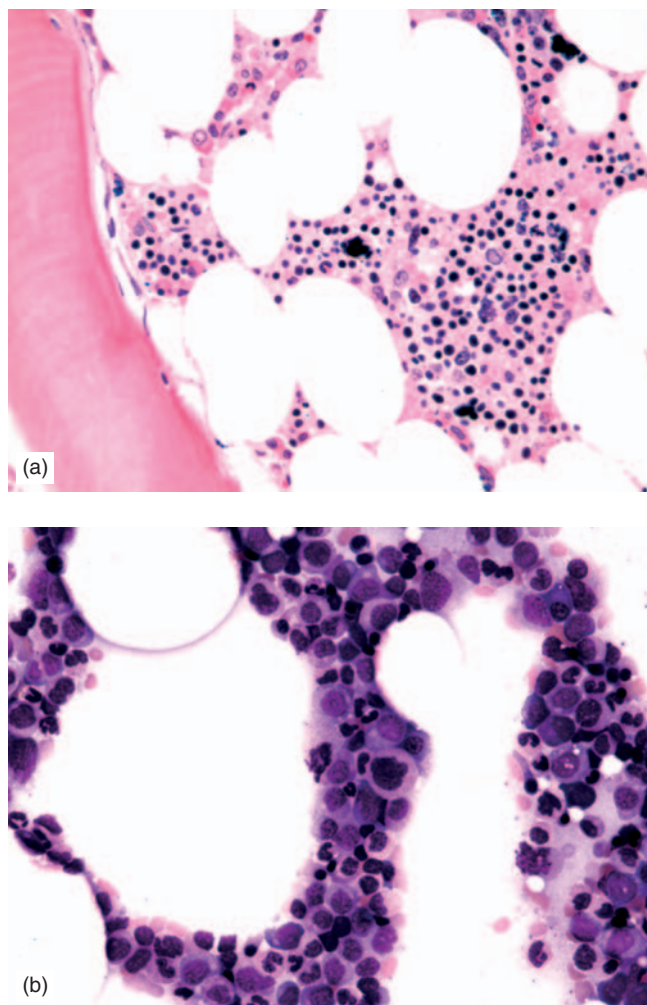


FIGURE 8.30 A bone marrow biopsy section (a) and a bone marrow smear (b) from a patient with hypocellular MDS.

immunosuppressive therapy. These patients are usually pancytopenic, show macrocytic anemia, with a variable marrow cellularity and dysplastic changes similar to those of classic MDS. Bone marrow blasts are under 5%.

However, as we mentioned earlier, about 10% of all MDS patients show clinical evidence of autoimmunity [22]. Because of this association, an autoimmune model has been suggested for pathogenesis of classic MDS, suggesting an autoimmune-induced apoptosis in this process.

HIV-Associated Myelodysplasia

Myelodysplastic changes observed in HIV-infected patients may be related to HIV, secondary infections or medications [85, 86]. Compared to classical MDS, patients with HIV-associated myelodysplasia more often show bone marrow hypocellularity, plasmacytosis, and eosinophilia [27, 87]. In these patients the degree of anemia and erythroid dysplasia is less severe, micromegakaryocytes are less frequent, blasts are <5%, no ringed sideroblasts are present, and cytogenetic studies are normal.

Paraneoplastic Myelodysplasia

Dysplastic changes similar to those of MDS have been reported in rare patients with solid tumors, such as carcinoma of colon, lung, kidney, prostate, and stomach [88–90]. The cause of dysplastic changes is not clear but does not appear to be drug-related. Production and release of growth factor-like proteins by the neoplastic cells is among the possibilities. Dysplastic changes are observed in both bone marrow and peripheral blood samples.

Myelodysplasia Associated with Heavy Metal Intoxication or Deficiency

There are occasional reports of myelodysplastic changes induced by arsenic and uranium intoxication, or copper deficiency [91, 92]. The dysplastic changes mimic RA or RCMD, and are often associated with anemia or pancytopenia. It is also interesting to know that arsenic trioxide, which acts through pro-apoptotic and anti-angiogenesis mechanisms, has been used to treat a variety of hematologic malignancies, including RAEB.

Myelodysplasia Associated with Chemotherapy or Irradiation

Myelodysplastic changes in bone marrow are common features of post-chemotherapy and radiotherapy. Bone marrow is hypocellular and shows multilineage dysplasia and myeloid left shift, mimicking hypocellular MDS [25–27].

CLINICAL ASPECTS

Clinical features of MDS represent bone marrow failure and cytopenia. Anemia, thrombocytopenia, and/or neutropenia may lead to symptoms such as fatigue, pallor, infection, bruising, and/or bleeding [1, 66–68]. But some patients may be asymptomatic at diagnosis. Establishment of the diagnosis is based on a multidisciplinary approach including morphologic evaluation and utilization of the accessory laboratory tests, such as immunophenotyping, cytogenetic analysis, molecular genetic studies, and *in vitro* colony growth assays [93].

Primary or *de novo* MDS is usually a disease of the elderly and is uncommon under the age of 50 years. The median age of onset is between 60 and 70 years with an estimated annual incidence of about 3.5–10 per 100,000 in the general population [1, 66–68]. t-MDS may arise at any age, usually 4–5 years after the initiation of chemotherapy or radiation therapy [72]. The percent of cytogenetic aberrations and risk of transformation to acute leukemia are significantly higher in t-MDS than in the primary MDS [59, 93]. A small proportion of MDS patients, roughly 4–5%, may develop blast transformation in extramedullary sites (granulocytic sarcoma), particularly skin. Evolution of MDS to granulocytic sarcoma is associated with poor prognosis.

According to the International Prognostic Scoring System (IPSS) for MDS patients (Tables 8.5 and 8.6), four distinctive risk groups are defined by low risk, intermediate

TABLE 8.5 The International Prognostic Scoring System (IPSS) for MDS.*

	Score			
	0	0.5	1.0	1.5
Prognostic variable				
% Blasts	<5	5–10	–	11–20
Karyotype**	Good	Intermediate	Poor	
Cytopenia(s)***	0–1	2–3		

*Adapted from Ref. [46].

Good: Normal, Y, del(5q); poor: complex (≥ 3 abnormalities), and chromosome 7 abnormalities; intermediate: other abnormalities.*Hemoglobin < 10 g/dL; Neutropenia < 1500/ μ L; Platelets < 100,000/ μ L.**TABLE 8.6** Survival and rate of transformation of MDS to acute leukemia according to the WHO subtypes.*

MDS subtype**	Median survival (years)	Evolution to AML (%)	IPSS score (%)***
RA	5.7	7.5	Low (57) Intermediate 1 (33) Intermediate 2 (10) High (0)
RARS	5.7	1.4	Low (96) Intermediate 1 (4) Intermediate 2 (0) High (0)
5q– syndrome	9.7	8	Low (61) Intermediate 1 (30) Intermediate 2 (9) High (0)
RCMD	2.7	10	Low (55) Intermediate 1 (40) Intermediate 2 (5) High (0)
RCMD-RS	2.6	13	Low (56) Intermediate 1 (36) Intermediate 2 (8) High (0)
RAEB-1	1.5	21	Low (0) Intermediate 1 (33) Intermediate 2 (55) High (12)
RAEB-2	0.8	34.5	Low (0) Intermediate 1 (0) Intermediate 2 (25) High (73)

*Adapted from Ref. [68].

**RA: refractory anemia; RARS: refractory anemia with ringed sideroblasts; RCMD: refractory cytopenia with multilineage dysplasia; RCMD-RS: refractory cytopenia with multilineage dysplasia and ringed sideroblasts; RAEB: refractory anemia with excess blasts.

***Percent in each category.

1 risk, intermediate 2 risk, and high risk [94, 95]. The major parameters measured in the IPSS are percent blasts, nature of the chromosomal aberrations, and the extent of cytopenia (Table 8.5). These four groups show different survival rates and carry various risk levels for transformation to acute leukemia. For example, the overall survival time for the low-risk group is about 5.7 years; intermediate 1 risk group approximately 3.5 years; intermediate 2 risk group about 1.2 years; and high-risk group approximately 0.4 years [94].

At the present time, the only effective therapy available for MDS is hematopoietic stem cell transplantation. Other promising, newly developed therapeutic approaches include the utilization of DNA methyltransferase inhibitors, vascular endothelial growth inhibitors, and the use of thalidomide, arsenic trioxide, and anti-TNF α [95–97].

DIFFERENTIAL DIAGNOSIS

Diagnosis of MDS is based on a multidisciplinary clinicopathologic approach. It is accomplished by obtaining adequate, pertinent clinical and environmental histories, careful pathologic review of peripheral blood and bone marrow, immunophenotyping, cytogenetic analysis, and molecular genetic studies. It should be noted that a broad spectrum of hematologic disorders may mimic MDS, and should therefore be considered in the differential diagnosis (Table 8.7).

Disorders with Dysplastic Erythropoiesis

Congenital dyserythropoietic anemias are hereditary disorders with bone marrow erythroid hyperplasia and marked dyserythropoiesis, such as megaloblastic changes, and the presence of erythroid precursors with bi- and multilobular nuclei (see Chapter 23). Bone marrow morphologic features of congenital dyserythropoietic anemias may mimic those of RA and RARS [98]. In congenital dyserythropoietic anemias, ringed sideroblasts are usually absent, myeloid and megakaryocytic lineages are unremarkable, and there is no abnormal karyotype.

Megaloblastic anemia may share morphologic features, such as erythroid dysplastic and megaloblastic changes, macrocytosis and neutrophilic hypersegmentation, with MDS. Megaloblastic anemia is characterized by low levels of serum folate or vitamin B12 and lack of ringed sideroblasts. A mild myeloid left shift may be seen in the bone marrow of some cases of megaloblastic anemia, but in such cases the blast cells are usually <5%. Cytogenetic and molecular studies are normal in megaloblastic anemia (see Chapter 23).

Disorders with ringed sideroblasts are seen in rare cases of hereditary sideroblastic anemia, and patients with pyridoxine deficiency, zinc or alcohol toxicity, or as post-medication effect in some patients treated with chloramphenicol, cycloserine, or anti-tuberculosis drugs.

TABLE 8.7 Differential diagnosis of MDS.

<i>Disorders with dysplastic erythropoiesis</i>	
Congenital dyserythropoietic anemias	Inherited disorders with marked erythroid dysplasia and unremarkable myeloid series and megakaryocytes. Normal karyotypes.
Megaloblastic anemia	Reduced serum levels of folate or vitamin B12. Normal karyotype.
Disorders with ringed sideroblasts	Observed in hereditary sideroblastic anemia (rare) and patients with pyridoxine deficiency, zinc or alcohol toxicity, or as post-medication effect in some patients treated with chloramphenicol, cycloserine, or anti-tuberculosis drugs.
Acute erythroleukemia	Erythroid lineage accounts for >50% of the bone marrow cells and myeloblasts make up $\geq 20\%$ of the non-erythroid component.
<i>Hyperplastic bone marrows with myeloid preponderance</i>	
Chronic myeloproliferative disorders	Peripheral blood cytosis, splenomegaly, no significant dyserythropoiesis, t(9;22) in CML, and JAK2 mutations in others.
Transient myeloproliferative disorder	A transient neonatal condition in Down syndrome; usually disappears in 4–6 weeks.
Acute myeloid leukemias	Blasts $\geq 20\%$; frequent balanced chromosomal aberrations.
<i>Hypoplastic bone marrows</i>	
Aplastic anemias	No increased blasts; no significant dysplastic changes; mutated genes in FA; mutated <i>PIG-A</i> gene in PNH with loss GPI-linked proteins (CD55, CD59).
Hypoplastic AML	Blasts $\geq 20\%$; frequent balanced chromosomal aberrations.
<i>Non-clonal myelodysplasia</i>	
	Observed in viral infections, autoimmune disorders, paraneoplastic syndromes, heavy metal intoxication, and post-chemotherapy and radiation therapy. Normal karyotype.

Erythroleukemia shares many morphologic features with RAEB and at times a distinction between these two entities is difficult. However, in erythroleukemia, the erythroid lineage accounts for >50% of the bone marrow cells and myeloblasts make up $\geq 20\%$ of the non-erythroid component (see Chapter 11).

Hyperplastic Bone Marrows with Myeloid Preponderance

Chronic myeloproliferative disorders (CMPD) may share overlapping bone marrow findings with some variants of MDS, such as 5q– syndrome, RCMD, and RAEB-1, either because of the presence of micromegakaryocytes or myeloid left shift. The accelerated phase of chronic myeloid leukemia (CML) may mimic RAEB-2. However, in CMPD, there is peripheral blood cytosis (thrombocytosis, granulocytosis, erythrocytosis, or all together), often with evidence of leukoerythroblastosis (presence of immature myeloid and erythroid cells in the blood) and no significant dysplastic changes. Splenomegaly is a frequent clinical presentation in myeloproliferative disorders but rare in MDS. As is discussed in Chapter 10, the bone marrow disorders under the WHO classification of myeloproliferative/myelodysplastic diseases show features of both CMPD and MDS.

Transient myeloproliferative disorder in Down syndrome is a neonatal condition which may mimic RAEB or AML with increased blasts in bone marrow and the presence of blasts in peripheral blood. This condition is transient and usually disappears within 4–6 weeks (see Chapter 22).

Acute myelogenous leukemias with relatively low blast counts (20–25%) are at times difficult to distinguish from RAEB-2, particularly when the blasts are dysplastic and do not fall into the classical morphologic criteria defined for normal blasts. Another challenge in estimating percent blasts is inadequate bone marrow aspirate smears (dry tap) due to fibrosis. In such cases, immunohistochemical stains for CD34 and CD117 may help to estimate the proportion of myeloblasts in the biopsy sections.

For the determination of percent blasts, the WHO recommends 500 and 200 differential counts for the bone marrow and blood smears, respectively [1]. Also, according to the WHO recommendation, disorders with evidence of known AML-associated cytogenetic abnormalities, such as t(8;21) and inv(16) should be considered as AML even if the blast count is <20%. Both MDS and AML show frequent cytogenetic abnormalities. While in MDS most aberrations represent unbalanced chromosomal aberrations (deletions, monosomies, and trisomies), AMLs often show balanced (reciprocal) chromosomal changes, such as translocations or inversions.

Hypoplastic Bone Marrows

Aplastic anemias, both acquired and constitutional forms, have overlapping features with hypocellular MDS. Aplastic anemia usually lacks significant dysplasia or evidence of increased blasts, but may occasionally show chromosomal aberrations. Most of the acquired aplastic anemias with cytogenetic abnormalities probably represent hypocellular MDS. Fanconi anemia is associated with FA complementary gene groups, and PNH shows evidence of mutations of the *PIGA* gene and loss of the expression of GPI-linked proteins, such as CD55 and CD59 on the hematopoietic cells (see Chapter 7).

Hypocellular AML shares many morphologic features with hypocellular MDS except for the higher percentage

($\geq 20\%$) of blast cells. Multilineage dysplasia is commonly found in hypocellular MDS, but it may be present or absent in hypocellular AML. Both lesions show frequent cytogenetic abnormalities.

Non-clonal Myelodysplasia

Non-clonal myelodysplastic changes are associated with conditions such as viral infections, autoimmune disorders, paraneoplastic syndromes, heavy metal intoxication, and post-chemotherapy and radiation therapy. These changes may be very similar to those of MDS. The clinical history and lack of chromosomal aberrations help to distinguish this broad entity from MDS.

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Chronic Myeloproliferative Diseases

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Chronic myeloproliferative diseases (CMPD), similar to myelodysplastic syndrome (MDS), are a group of hematologic disorders distinguished by clonal expansion of abnormal hematopoietic stem cells at different levels leading to a hypercellular marrow with excessive terminal proliferation of the hematopoietic cells and peripheral blood granulocytosis, erythrocytosis, and/or thrombocytosis. This hyperproliferative process, in certain conditions, is associated with bone marrow fibrosis and extramedullary hematopoiesis. The extramedullary hematopoiesis along with excess sequestration of the hematopoietic cells in the spleen often leads to massive splenomegaly, one of the clinical hallmarks of CMPD. Hepatomegaly, though less frequent, may be also present [1–6a].

Morphologic features shared by various types of CMPD include (Figure 9.1):

2. Tear-drop-shaped erythrocytes.
3. Giant platelets.
4. Frequent basophilia and/or eosinophilia.
5. Lack of significant dysgranulopoiesis.
6. Lack of toxic granulation in neutrophils.

The classification of CMPD by the World Health Organization (WHO) includes the following categories [1]:

1. Chronic myelogenous leukemia (CML).
2. Chronic neutrophilic leukemia (CNL).
3. Chronic eosinophilic leukemia (CEL) and hypereosinophilic syndrome (HES).
4. Polycythemia vera (PV).
5. Chronic idiopathic myelofibrosis (with extramedullary hematopoiesis) (CIMF).
6. Essential thrombocythemia (ET).
7. Chronic myeloproliferative disease, unclassifiable (CMPD-U).

The category of *mast cell diseases* has been added into the above classification in a recently revised draft of the WHO classification (updated WHO classification in press [6b]). In this book, mast cell disorders are discussed separately in Chapter 20.

The exact mechanisms of the lymphoproliferative process in these disorders are not well understood. The hypersensitivity of the affected stem cells to certain growth factors and/or defective negative regulatory feedback mechanisms may play a role. However, recent investigations suggest abnormalities in tyrosine kinase genes as the central core to the pathogenesis of CMPD. The classical example of tyrosine kinase involvement in CMPD is the fusion of *ABL1* and *BCR* genes t(9;22)(q34;q11.2); the Philadelphia chromosome] in chronic

Bone Marrow

1. Hypercellular bone marrow with mono- or multilineage hyperplasia, predominance of mature cells, and no significant dyserythropoiesis or dysgranulopoiesis.
2. Megakaryocytosis, often present in clusters with abnormal morphology.
3. Dilated sinuses containing clusters of hematopoietic cells.
4. Frequent focal or diffuse marrow fibrosis.
5. Frequent osteosclerosis.
6. Frequent basophilia and/or eosinophilia.

Blood

1. Granulocytosis, erythrocytosis, and/or thrombocytosis, often with a leukoerythroblastic picture.

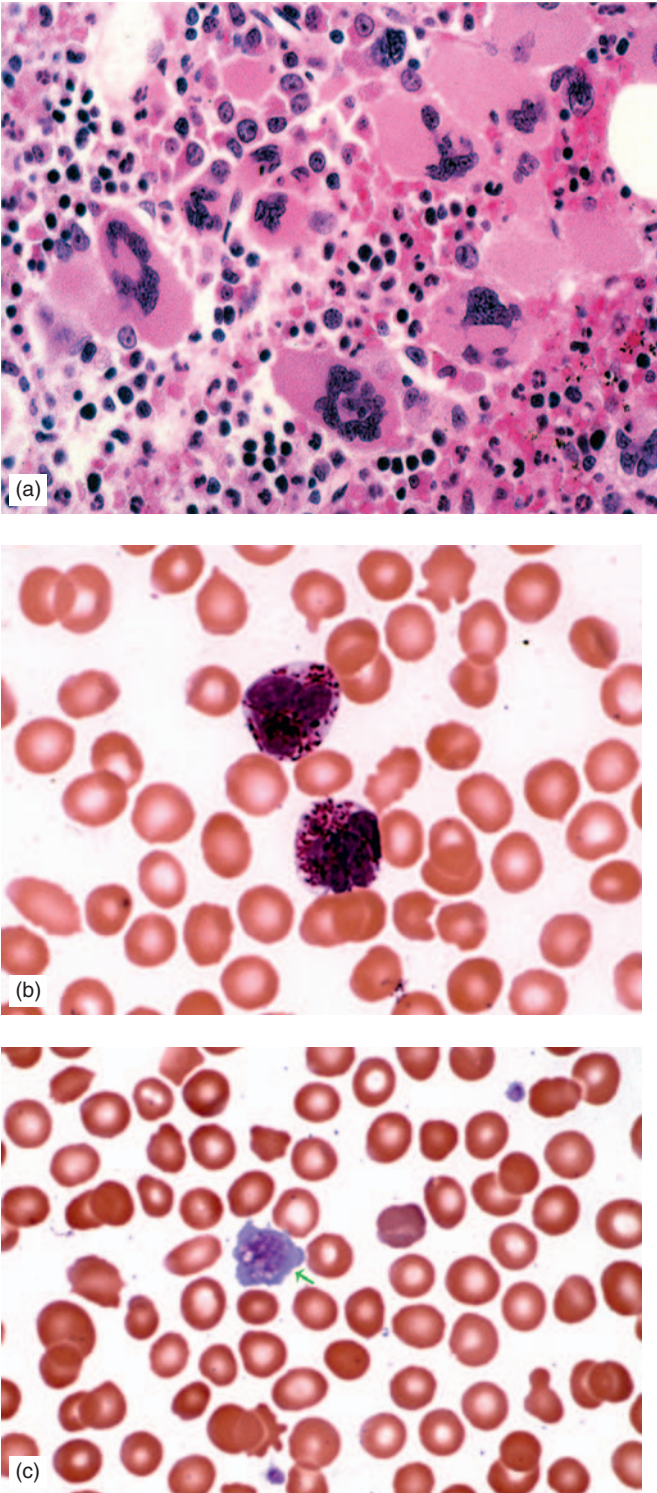


FIGURE 9.1 Chronic myeloproliferative disorders share some morphologic features such as (a) bone marrow hypercellularity and increased megakaryocytes with atypical features, (b) basophilia, and (c) the presence of giant platelets (arrow).

myelogenous leukemia. Several tyrosine kinase genes, other than *ABL1*, have been identified, such as *ABL2*, *PDGFRA*, *PDGFRB*, *FGFR1*, and *JAK2*. The fusion of *FIP1L1A-PDGFR1* genes and the activating V617F mutation in the

TABLE 9.1 Chromosomal aberrations in chronic myeloproliferative disorders.

Chromosome abnormality	Frequency (%)	Prognosis
Trisomy 1q	8	
4q12 deletion (CHIC2)		Response to Imanitib
5q33 aberrations (PDGFB)		Response to Imanitib
Monosomy 7	5	
Trisomy 8	16	Good prognosis
8p11 translocations		Poor prognosis
Trisomy 9 or 9p	10	Unclear
12p aberrations	3	
13q deletion	7	Unclear to good
20q deletion	9	Good

TABLE 9.2 Incidence of chromosomal abnormalities in subtypes of chronic myeloproliferative disorders.

Subtype*	Frequency (%)
CML	95
PV	34
CIMF	40
HES	7–12
ET	<3

*CML: chronic myelogenous leukemia, PV: polycythemia vera, CIMF: chronic idiopathic myelofibrosis, HES: hypereosinophilic syndrome, and ET: essential thrombocythemia.

JAK2 gene have been recently reported in a significant proportion of Philadelphia-negative CMPD cases [7–9].

The spectrum of cytogenetic aberrations in CMPD is heterogeneous, ranging from numerical gains and losses to structural changes including unbalanced translocations (Table 9.1) [10, 11]. Chromosomal gains and losses rather than balanced translocations appear to be common in CMPD. Standard karyotyping along with FISH is important in establishing the diagnosis and may provide very useful information for disease outcome.

Cytogenetic abnormalities in myeloproliferative disorder (MPD) subtypes other than CML occur at different frequencies ranging from 3% to 40%, depending on the subtype (Table 9.2). Compared to CML, the other MPD subtypes are more clinically and cytogenetically heterogeneous. In fact, at least 27 different chromosomal anomalies have been associated with MPD. Unlike the “Philadelphia chromosome” in CML, there is no pathognomonic chromosomal abnormality associated with the MPDs.



FIGURE 9.2 A G-banded karyotype with trisomy 9.

Chromosomal abnormalities are seen in 30–40% of patients with PV and IMF and seem to indicate a poor prognosis. On the other hand, chromosomal abnormalities are rare in patients with ET (about 5–6%). In cases suspicious for ET, cytogenetic studies are used for exclusion of other hematological malignancies associated with increased megakaryopoiesis such as 5q-syndrome or AML with inversion chromosome 3q.

Consistent acquired changes seen at diagnosis include deletion of the long arm of chromosome 20, del(13q), trisomy 8 and 9, and duplication of parts of 1q (Figures 9.2 and 9.3). Furthermore, del(20q), trisomy 8, and dupl(1q) all arise in multipotent progenitor cells. The molecular mapping of 20q deletions and, to some extent, 13q deletions has identified a number of candidate target genes, although no mutations have yet been found. Finally, translocations associated with the rare 8p11 myeloproliferative syndrome (Figure 9.4) and other atypical MPDs have permitted the identification of a number of novel fusion proteins involving fibroblast growth factor receptor-1 (*FGFR1*). Chromosomal anomalies are found most frequently in chronic idiopathic myelofibrosis, CIMF (up to 50%), followed by PV, whereas anomalies in ET and CEL are so infrequent that cytogenetics can be omitted when the diagnosis is clear [11–13]. The most common structural chromosomal anomalies of MPD in order of frequency are t(9;22)(q34;q11.2), del(20)(q11q13), del(13)(q12q14), del(5)(q13q33), and del(12)(p12). The most common numeric anomalies are loss of Y, +8, +9, and 7. Only the t(9;22) (or variant 9;22) is diagnostic of any specific type of MPD (CML). Relatively strong associations are observed for the del(13) in CIMF, the t(5;12)(q33;p13) in CEL, and the del(20), +8, and +9 in PV [11, 13]. Balanced translocations are rare [10, 11]. Molecular cytogenetic techniques in CMPD have suggested that some abnormalities may be more common than originally thought, whereas molecular studies are likely to

detect the possible role of candidate genes implicated in the neoplastic process [11, 13–22].

The observation of a subclone or stem line with multiple chromosomal anomalies is often an indication of disease progression or clonal evolution [23]. This evolution is seen in at least 12% of patients with MPD. Multiple clones have been observed in 2.1% of patients with hematologic malignancies and in 1.8% of patients with MPD. The observation of multiple clones is most common among patients with a clone harboring t(9;22), del(20q), or +8 [13].

CHRONIC MYELOGENOUS LEUKEMIA

Also referred to as chronic myeloid, myelocytic, or granulocytic leukemia, CML was the first malignant disorder reported in association with a chromosomal aberration, the Philadelphia chromosome (*Ph^t*). CML has been the front runner in the understanding of molecular mechanisms in hematopoietic malignancies and target treatment approaches [24–26]. It is characterized by clonal expansion of bone marrow stem cells leading to selective granulocytic hyperplasia with or without thrombocytosis and the presence of its cytogenetic hallmark *Ph^t*, which is the result of balanced reciprocal t(9;22)(q34;q11.2) chromosomal translocation, resulting in a short 22q (*Ph^t*). CML demonstrates an evolutionary process with different clinicopathological stages of *chronic*, *accelerated*, and *acute* (blast transformation) phases.

Etiology and Pathogenesis

Ionizing radiation has been reported as a possible inducing agent in the development of CML based on the observation of increased incidence of CML 5 to 10 years after radiation

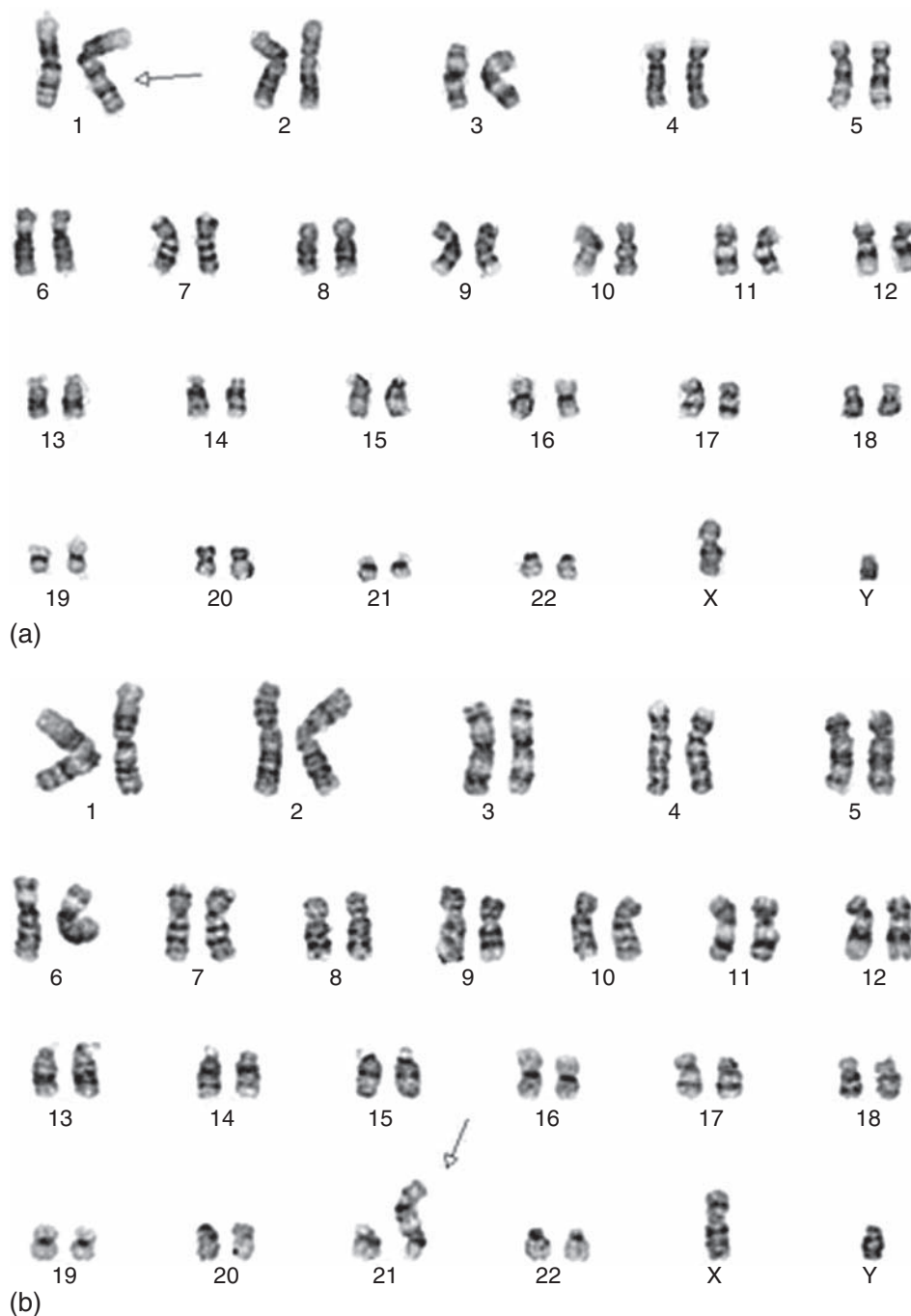


FIGURE 9.3 Duplication of 1q: (a) interstitial duplication of 1q and (b) whole arm duplication of 1q.

exposure. Also, possibility of a susceptibility gene has been raised based on the increased frequency of HLA-Cw3 and -Cw4 in CML patients, though hereditary disposition does not seem to play a significant role [27, 28]. Only very rare familial childhood forms have been reported [29, 30].

The pathognomonic hallmark for CML, as mentioned, is $t(9;22)(q34;q11.2)$ (Ph^1), which is observed in hematopoietic cells but not in bone marrow stromal cells. This chromosomal translocation creates a *BCR-ABL1* fusion gene with three different principal protein products, based on the site of the breakpoint on chromosome 22. All three *BCR-ABL1* protein products (p190, p210, and p230) demonstrate

increased tyrosine kinase activity and are not detected in normal hematopoietic cells. A wide variety of pathogenic effects have been contributed to the *BCR-ABL1* gene fusion products including [26]:

1. Insensitivity of Ph^1 positive hematopoietic progenitor cells to growth-inhibiting regulatory cytokines.
2. Mitogenic activity of the *BCR-ABL1* fusion proteins on hematopoietic cells.
3. Resistance of Ph^1 positive hematopoietic cultured cell lines to apoptosis. The *in vitro* reports regarding this matter remain controversial.



FIGURE 9.4 A G-banded karyotype with an 8;22 translocation involving the 8p11 band and 22q11.2 (red arrows).

4. Decreased adherence of *Pb1* positive hematopoietic progenitors to bone marrow stroma and fibronectin, leading to increased circulation of myeloid cells.
5. Genetic instability of *Pb1* positive hematopoietic progenitors leading to progression from a chronic phase to blast crisis.
6. Ability of the *BCR-ABL1* fusion proteins to promote leukemogenesis in mice.

Pathology

Morphology and Laboratory Findings

Bone marrow sections are hypercellular with marked myeloid preponderance and mild to moderate myeloid left shift (Figure 9.5). The paratrabecular myeloid regions are expanded with less mature forms next to the bone trabeculae and more mature forms closer to the center. Eosinophilia is a frequent finding. Megakaryocytes are usually increased and often appear in clusters. There may be patchy or diffuse fibrosis along with osteosclerosis. The extent of fibrosis to some degree correlates with the number of megakaryocytes. Scattered or clusters of pseudo-Gaucher cells (histiocytes) with abundant wrinkled cytoplasm are often present (Figure 9.5c) [1–4, 31, 32].

Bone marrow smears are highly cellular with an elevated M:E ratio of usually >10:1. Eosinophils are increased, and basophilia is a frequent feature but is usually <20% (Figure 9.6). Dysgranulopoiesis may be present but is not prominent. There may be mild to moderate myeloid left shift, but myeloblasts are usually below 5%. Megakaryocytes often show dysplastic changes, including the presence of numerous micromegakaryocytes (“dwarf” forms) as well as many large, bizarre, multilobulated forms,

individually or in clusters. Pseudo-Gaucher cells are usually found attached to or in the vicinity of the stromal tissue fragments. Sometimes, their cytoplasm appears light blue by Wright’s stain; hence referred to as “sea-blue histiocytes” (Figure 9.6b). Sea-blue histiocytes and pseudo-Gaucher cells are loaded with phagocytic particles and cell membrane debris due to the increased turnover of the bone marrow cells [1–4, 31, 32].

Blood smears show marked leukocytosis with a white blood cell count of often >100,000/ μ L. The morphologic findings of the myeloid cells mimic bone marrow with the presence of myeloid left shift and a wide spectrum of myeloid precursors, including myeloblasts and promyelocytes (Figure 9.7). Similar to in the bone marrow smears, myeloblasts in blood smears are usually below 5%. Usually, myelocytes are more numerous than metamyelocytes (myelocyte bulge). Absolute basophilia and eosinophilia is common, and there may be absolute monocytosis, but the monocytes in differential count usually do not exceed 3%. Scattered nucleated red blood cells may be present. Platelet count is normal or elevated but occasionally reduced during the chronic phase. Neutrophils and bands show reduced alkaline phosphatase activity by cytochemical stains [known as “leukocyte alkaline phosphatase (LAP) score”]. The elevated levels of serum lactic dehydrogenase, uric acid, and vitamin B₁₂ are also frequently observed [1–4, 31, 32].

Splenomegaly is common. The red pulp is diffusely expanded with infiltration of the cords and sinuses filled with mature and immature myeloid cells (Figure 9.8). The malpighian corpuscles (white pulp) are reduced in size and number or are completely absent [33].

The recent implementation of effective targeted therapy in CML, such as treatment with imatinib, has created a necessity for follow-up bone marrow and molecular studies. The evaluation of post-therapy bone marrow samples shows

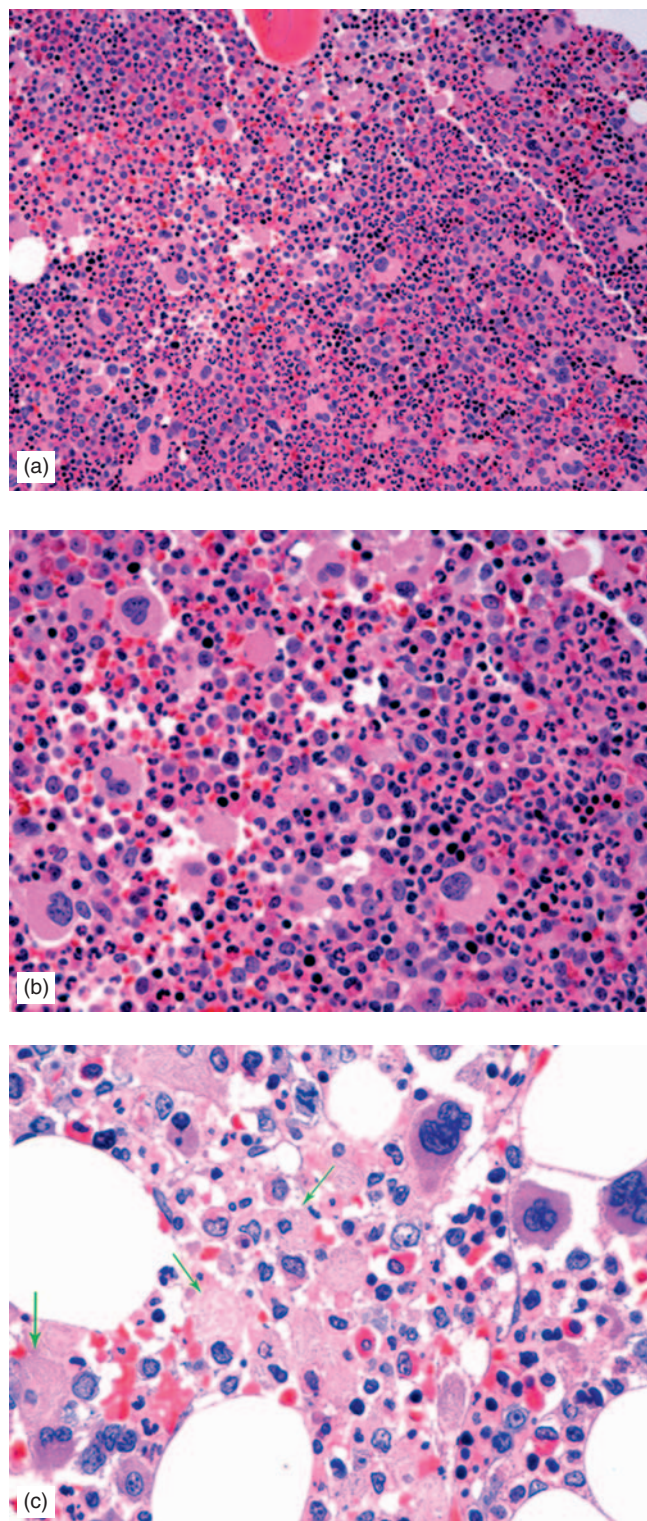


FIGURE 9.5 Bone marrow biopsy sections in patients with CML are hypercellular, show marked myeloid preponderance and increased megakaryocytes with small forms: (a) low power and (b) high power. A small aggregate of histiocytes (pseudo-Gaucher cells) is demonstrated in (c) (arrows).

progressive changes toward normal morphology. However, the post-therapy bone marrow samples may show certain morphologic features such as:

1. Frequent presence of non-diagnostic lymphoid aggregates, sometimes paratrabecular, consisting of a mixture of B and T lymphocytes.
2. Frequent presence of histiocytic aggregates (pseudo-Gaucher cells).
3. Bone marrow hypocellularity, particularly in cases with long history of treatment. The degree of hypocellularity in some instances is so severe that the bone marrow biopsy sections resemble aplastic anemia.

Accelerated phase of CML is often associated with a decline in the patient's clinical condition along with certain laboratory findings. The diagnosis of CML in accelerated phase (CML-AP), according to the WHO recommendation, is based on the presence of one or more of the following (Table 9.3) (Figure 9.9) [1]:

1. The presence of 10–19% blasts in blood or bone marrow samples.
2. Basophilia of $\geq 20\%$.
3. Persistent thrombocytopenia of $\leq 100,000/\mu\text{L}$ or thrombocytosis of $\geq 1,000,000/\mu\text{L}$.
4. Progressive splenomegaly and/or increasing leukocyte count.
5. Cytogenetic or molecular evidence of clonal evolution.

Increased marrow fibrosis, marked megakaryocytosis with the presence of large clusters or sheets of megakaryocytes, and severe dysgranulopoiesis are all suggestive of CML-AP. CML-AP is a transient phase between the chronic phase and blast transformation [34].

Blast transformation (blast crisis) refers to the evolution of CML into acute leukemia. The exact mechanisms involved in this evolutionary process are not well understood, but recent studies suggest that the unrestricted activity of the *BCR-ABL1* fusion gene may play an important role [34–36]. Enhanced proliferation and differentiation arrest, the characteristic features of blast transformation in CML, seem to be dependent upon the cooperation of *BCR-ABL1* with the *p53* (17p13) and *RB1* (13q14) genes that appear to be directly or indirectly dysregulated in this process.

According to the recommendation of WHO, the diagnosis of CML in blast crisis (CML-BC) is made when (Table 9.3) (Figures 9.10 and 9.11) [1]:

1. Blasts are $\geq 20\%$ of bone marrow nucleated cells or peripheral blood differential count.
2. Large foci or clusters of blasts are present in the bone marrow biopsy sections.
3. There is evidence of extramedullary tissue infiltration by blast cells.

CML-BC represents all morphologic features required for the diagnosis of acute leukemia. Blasts are $\geq 20\%$ of the bone marrow smear or blood smear differential counts or appear in sheets or large clusters in the bone

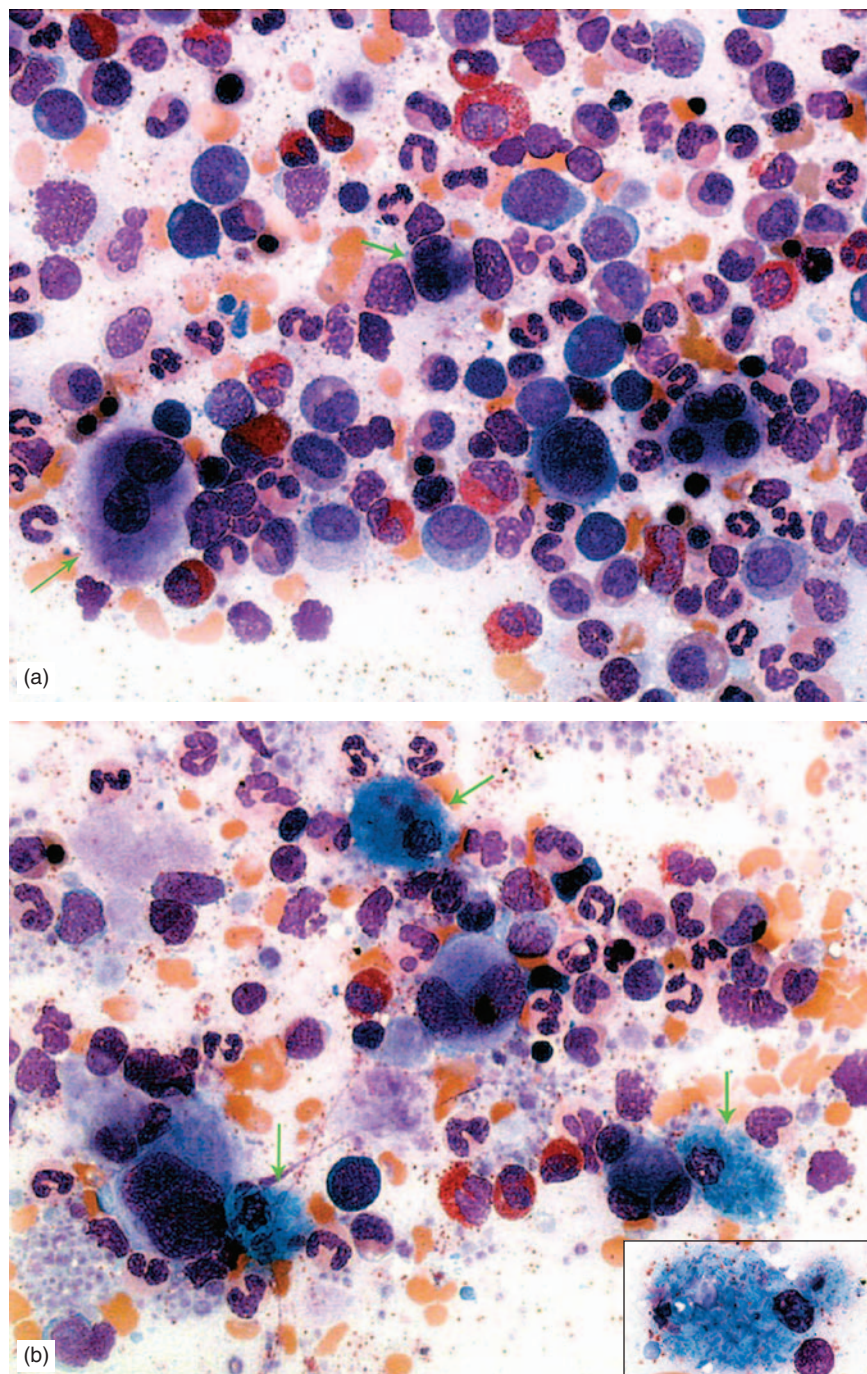


FIGURE 9.6 Bone marrow smears in a patient with CML showing myeloid preponderance, eosinophilia, and the presence of small megakaryocytes (a, arrows). Several sea-blue histiocytes are demonstrated (b, arrows and inset).

marrow biopsy and/or clot sections. There may be some degree of dysmyelopoiesis or bone marrow fibrosis.

Extramedullary CML-BC may involve any tissue, but is frequently observed in spleen, lymph node, skin, and central nervous system. Morphologic features are similar to other acute leukemic infiltrations. There is often the presence of immature eosinophils (eosinophilic myelocytes) which may provide a hint that blasts are of myeloid lineage.

The blast cells in approximately 70% of CML-BC cases are of myeloid (non-lymphoid) origin and express granulocytic, monocytic-, erythroid-, and/or megakaryocytic-associated CD molecules in immunophenotypic studies [35]. In roughly 30% of the CML cases, blast

transformation is of lymphoid lineage. In our experience, a significant proportion of CML-BC cases consist of myeloblasts with the aberrant expression of lymphoid-associated CD molecules or blasts with biphenotypic features.

Immunophenotypic Studies

Flow cytometry is primarily used in CML-AP and CML-BC for the estimation of blast counts and their lineage assignments. However, there is some evidence of abnormal expression of CD molecules on mature and immature myeloid cells in patients with CML in chronic phase (CML-CP). For example, there are reports of reduced density of CD16

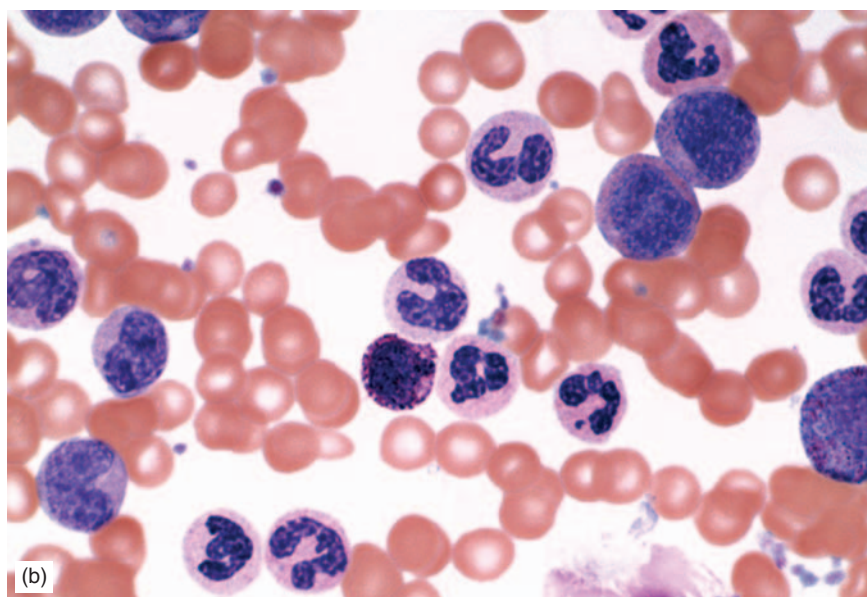
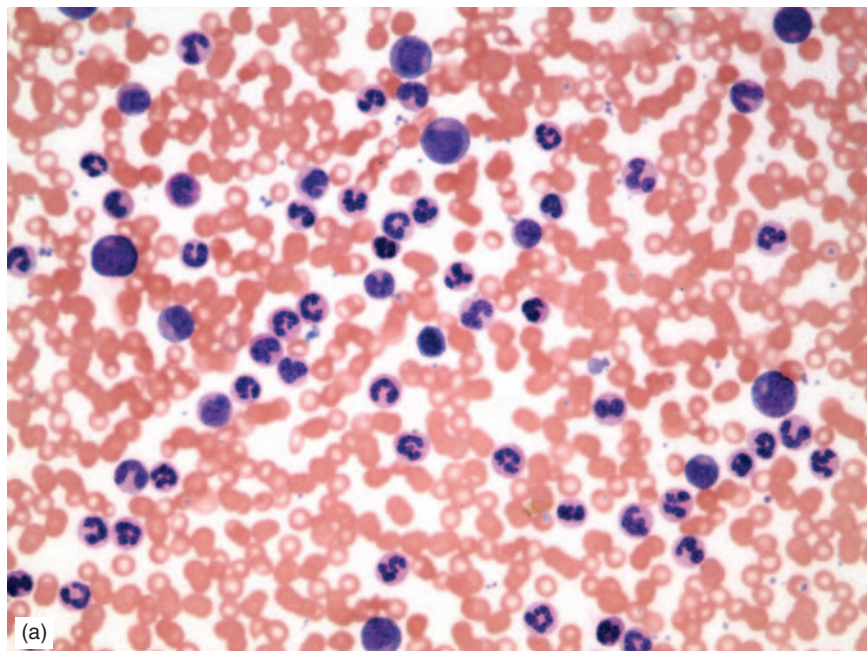


FIGURE 9.7 Peripheral blood smears of patients with CML show marked leukocytosis with myeloid left shift and high proportion of myelocytes and metamyelocytes (a) and (b); basophils are often present (b).

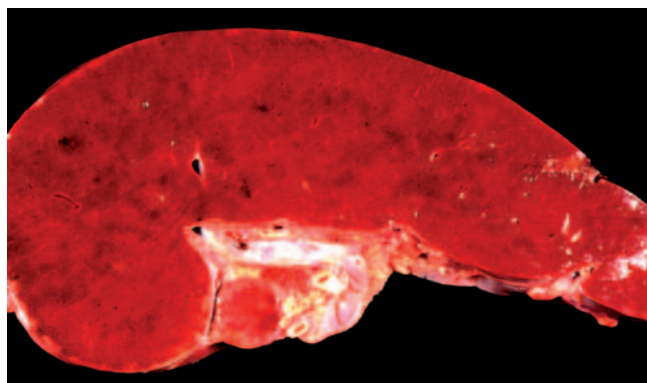


FIGURE 9.8 Splenomegaly is a frequent clinical finding in CML. Splenic involvement is usually diffuse.

expression on the neutrophils and an increased proportion of CD7+ myeloid stem cells (CD34+, CD33+) in patients with CML-CP [37].

A few flow cytometric studies have shown a significant reduction in the average telomere length of leukocytes in CML-AP and CML-BC patients. Also, there is a report of higher S-phase values in the leukocytes of patients with CML-AP (9 ± 3) than patients with CML-CP (5 ± 2) or normal controls ($<1\%$) by flow cytometry [38]. In this study, the CML-CP cases with S-phase values of $>7\%$ evolved into accelerated phase (AP) within 18 months. It is possible to estimate the basophil counts in the bone marrow or the peripheral blood by looking at the CD45^{dim}, cells that in addition to CD13 and CD33 express CD22.

Blasts in AP and blast crisis of CML are often of myeloid origin ($>70\%$ of the cases) and in order of frequency

TABLE 9.3 Evolution of chronic myelogenous leukemia (CML).*

Stage	Characteristics
<i>Chronic phase</i>	Leukocytosis (often $>100,000/\mu\text{L}$) Hypercellular marrow with marked myeloid preponderance Blasts $<10\%$ Basophilia $<20\%$ Eosinophilia Megakaryocytosis with the presence of micromegakaryocytes Splenomegaly Low LAP score $t(9;22)(q34;q11.2)$ <i>BCR-ABL1</i> fusion by FISH and/or RT-PCR
<i>Accelerated phase</i>	Hypercellular marrow with myeloid left shift Blasts $10\text{--}19\%$ Basophilia $\geq 20\%$ Thrombocytopenia $<100,000/\mu\text{L}$, or thrombocytosis $>1,000,000/\mu\text{L}$ Increasing WBC count Increasing spleen size Additional cytogenetic abnormalities
<i>Blast crisis (phase)</i>	Blasts $\geq 20\%$ Sheets or large clusters of blasts in the bone marrow biopsy Extramedullary tissue infiltration by blast cells

*Adapted from Ref. [1].

express CD33, CD13, CD11c, CD36, CD34, CD117, and CD15. Lymphoid blast transformation is usually of precursor B with the expression of TdT, CD19, CD10, and less frequently, CD20. Precursor T blast transformation is a rare event. As mentioned earlier, not infrequently, CML blasts show aberrant expression of CD molecules or evidence of biphenotypic features (see Chapter 13) [39, 40]. In our experience, the most frequent lymphoid-associated markers aberrantly expressed on myeloblasts are CD7, CD56, and CD19, and the most frequent myeloid-associated markers aberrantly expressed on lymphoblasts are CD15, CD13, and CD33.

Immunohistochemical stains are helpful for the estimation of blast counts and identification of their lineage, particularly when there is no access to flow cytometry or there is inadequate marrow aspirate (dry tap). The following markers are frequently used: CD34 (blasts); CD117 (myeloblasts); TdT (lymphoblasts); CD31, CD61, and factor VIII for megakaryoblasts; glycophorin A and hemoglobin A for erythroid precursors; myeloperoxidase; lysozyme and CD68 for granulocytic and monocytic lineages; CD10, CD20, and CD79a for B lymphocytes; and CD2, CD3, CD5, and CD7 for T lymphocytes.

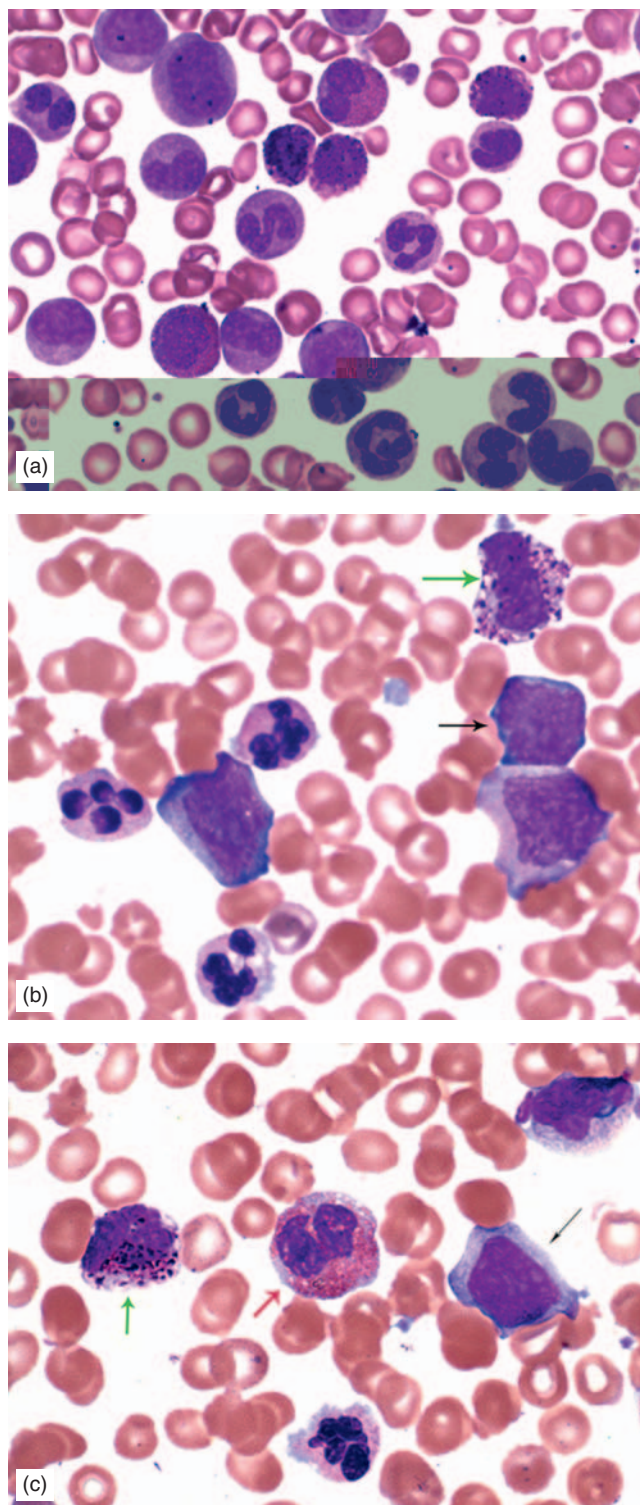


FIGURE 9.9 Peripheral blood smear of a patient with CML-AP showing myeloid left shift (a, intermediate power), basophils (green arrows) and blasts (black arrows) (b and c, high power). An eosinophil (red arrow) is demonstrated in (c).

Molecular Studies

As noted earlier, detection of the *BCR-ABL1* fusion gene is the hallmark of CML in diagnosis, monitoring, and targeted therapy. While traditionally this has been done by cytogenetic

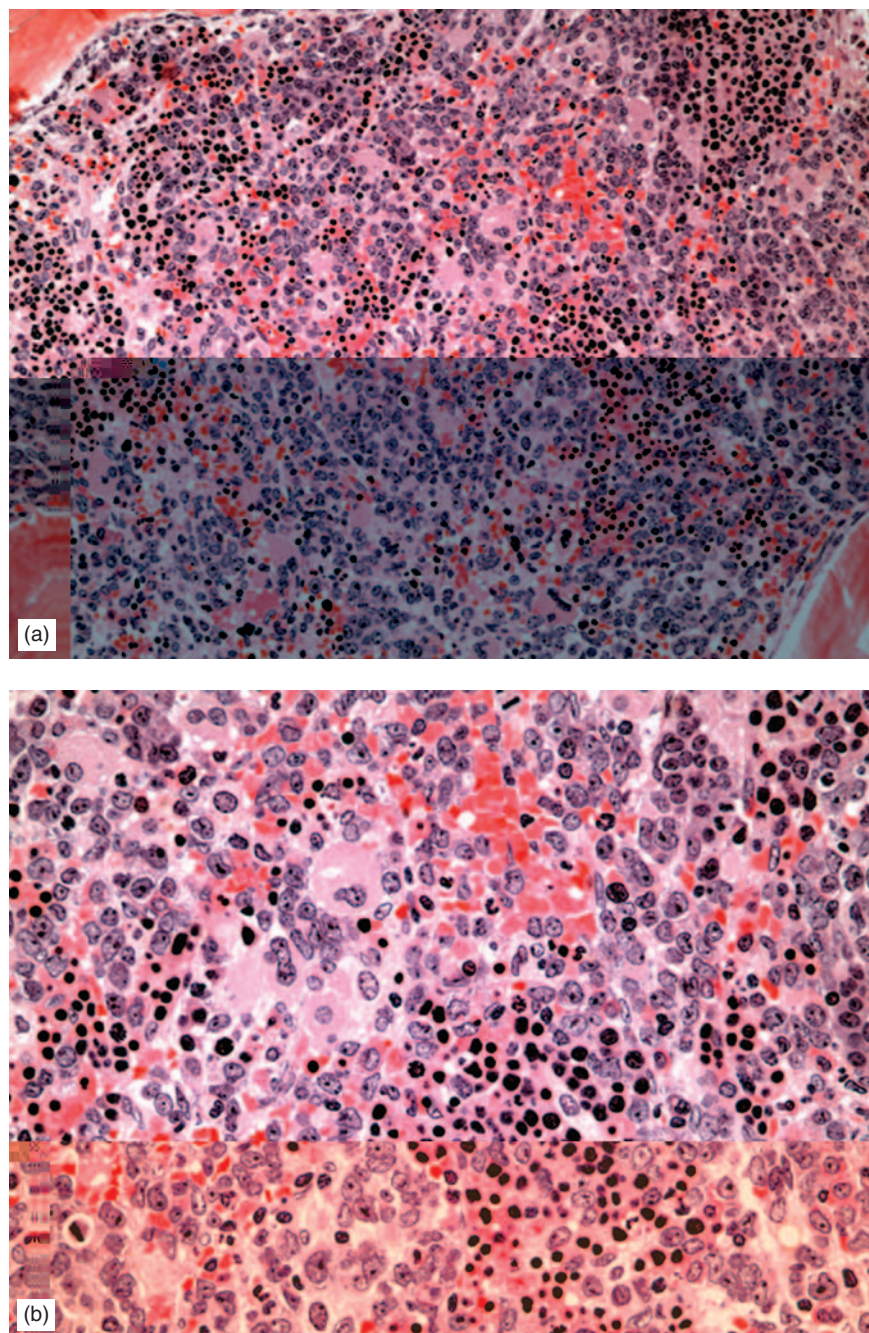


FIGURE 9.10 Bone marrow biopsy section of a patient with a history of CML showing increased blasts consistent with blast transformation: (a) low power and (b) high power.

analysis, and more recently molecular cytogenetic (FISH) testing, the most sensitive and quantitative approach is by molecular methods. The sensitivity of classical cytogenetics is limited by the number of cells cultured to the number of metaphases counted. Moreover, about 5% of CML cases have t(9;22) translocations that may not be visible under the light microscope and will thus be missed by this approach. Molecular testing, by any one of a number of available methods, should be able to detect such “cryptic” translocations and will therefore approach 100% sensitivity for initial diagnosis [41].

The translocation of the *BCR* gene on chromosome 22 to the *ABL1* oncogene on chromosome 9 produces a fusion protein with constitutive tyrosine kinase activity which is much higher than that found in normal myeloid cells. *BCR* stands for “breakpoint cluster region,” reflecting the fact that the point of breakage at that site can occur over a fairly broad region. While many subtle variants are possible, the important rearrangements for clinical diagnosis are the major breakpoint (*M-BCR*) which produces the p210 gene product and is found primarily in CML and the minor breakpoint (*m-BCR*) which produces the p190 gene product and is

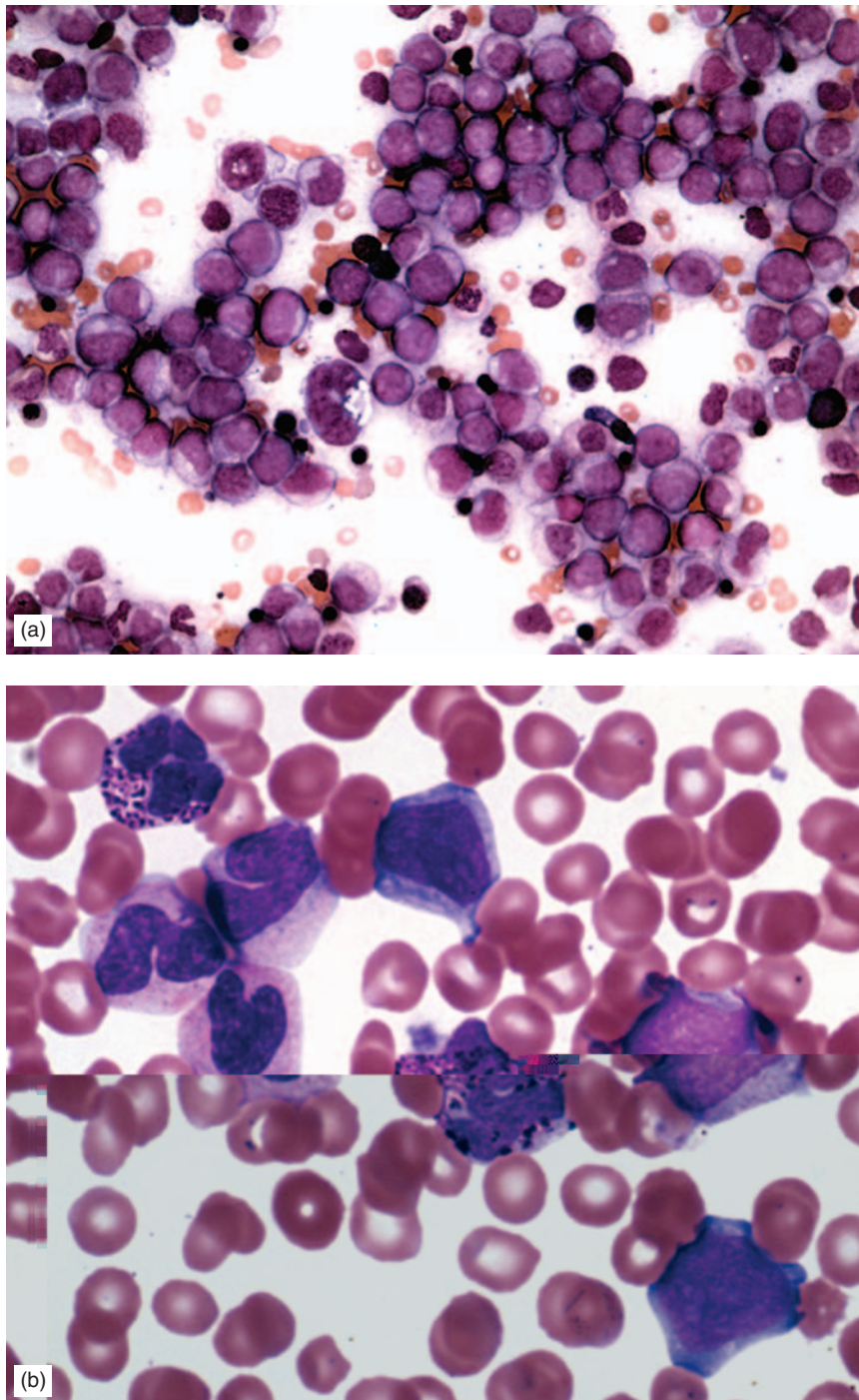


FIGURE 9.11 Bone marrow (a) and blood (b) smears of a patient with CML in blast transformation.

typical of acute lymphoblastic leukemia, more commonly seen in adult patients with that disease. However, some overlap is seen in a minority of cases, owing to alternative splicing of RNA transcripts [42]. One clear advantage of molecular testing is that it can readily distinguish between these isoforms.

The first molecular test for the *BCR-ABL1* translocation to enter wide use was the Southern blot, in which genomic DNA extracted from the patient's blood or bone marrow cells is digested with restriction endonucleases, subjected to agarose gel electrophoresis, blotted onto a nylon filter,

and hybridized with a radioactively labeled DNA probe complementary to the *BCR* gene. The principle behind the assay is that a translocation of *BCR* on chromosome 22q11.2 to chromosome 9q34 (*ABL1*) will place it in a different milieu of restriction enzyme cleavage sites, producing a band shift on the resulting autoradiogram. The anomalous band(s) may be of higher or lower molecular weight than the unrearranged (germline) bands; in most cases the latter will also be seen because of the presence of non-malignant cells in a mixed specimen and/or the retention of one of the

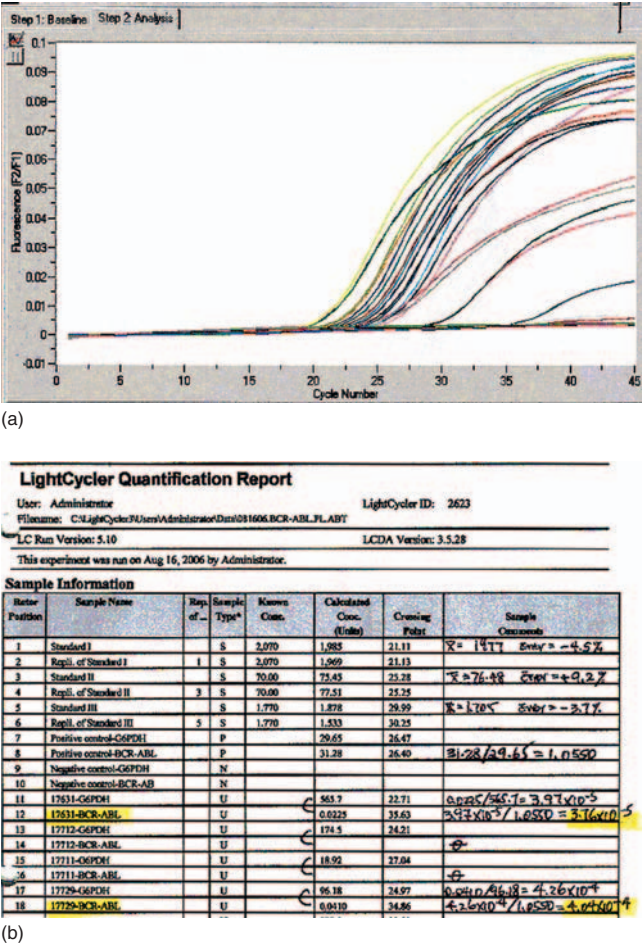


FIGURE 9.12 Data obtained from real-time PCR analysis of a series of patients with suspected, diagnosed, or treated CML using the LightCycler instrument (Roche Molecular Diagnostics, Indianapolis, IN). (a) Amplification curves produced from extracted cellular mRNA that was first reverse transcribed to create cDNA templates; the specimens producing curves that enter log phase at lower PCR cycle number had higher amounts of starting *BCR-ABL* fusion mRNA. (b) Quantitative readout of data from the LightCycler; specimens that are *BCR-ABL*-negative show no amplification product from this target (but do show output from the control *GAPDH* gene target), whereas those specimens that are *BCR-ABL*-positive show amplification products from both genes. Quantitation of the *BCR-ABL* fusion gene is done mathematically by comparing its amplification to that of the *GAPDH* control gene.

BCR alleles in the germline configuration even in the CML cells. By convention, two different restriction enzymes are used, and extra bands must be observed with both digests in order to be diagnostic; an extra band seen with only one of the enzymes could represent a benign restriction fragment length polymorphism. While useful and more sensitive than classical cytogenetic testing, the Southern blot has a number of disadvantages in this context: (1) it is laborious, time-consuming, and expensive; (2) it utilizes large amounts of hazardous radioisotopes for probe labeling (at least until the more recent advent of chemiluminescent probe labeling systems); (3) it is not very useful for detecting minimal residual disease because the bands produced by *BCR-ABL1*-positive cells present at <5% of the total cell

population may be too faint to be visible; and (4) it is generally not capable of distinguishing between the major and minor breakpoint forms, unless highly specific DNA probes are used.

Because of these disadvantages, most laboratories eventually moved on to methods based on polymerase chain reaction (PCR). If one of the PCR primers is chosen to hybridize at the *BCR* site and the other at the *ABL1* site, an amplified product will be seen only if the two genes are fused, bringing the two primers into proximity. Otherwise, a completely blank result will be produced, because single PCR primers hybridized to widely distant (in this case, on different chromosomes) regions of the genome are incapable of supporting the exponential amplification needed to visualize a product. One catch of this approach is that the breakpoint region on chromosome 22 spans such a large area that it cannot be efficiently amplified from a genomic DNA target. Instead, messenger RNA must be isolated to serve as the template; since it has had the large intronic regions spliced out, it is of an amplifiable size. Most laboratories now use a real-time PCR system that is both highly sensitive and highly quantitative. It is also capable, depending upon how the primer pairs are chosen, of differentiating between the *M-BCR* and *m-BCR* forms, which can be of great importance in the differential diagnosis (in a newly ascertained patient) of acute leukemia versus the blast crisis phase of CML [43]. Examples of the data produced by such systems are shown in Figure 9.12. Some test systems generate absolute quantitation (e.g. in nanograms) of *BCR-ABL1* fusion gene, whereas others, such as that shown here, produce a relative quantitation by comparing *BCR-ABL1* to an internal control gene (in this case, *GAPDH*).

In our hands, the real-time PCR method is sensitive down to a level of 1 CML cell in 1 million normal cells. It is also highly quantitative, so that trends (upward or downward) in treated patients obtained from periodic monitoring can be used to assess minimal residual disease, relapse, or development of resistance to the newer pharmacogenetic therapies (see later) [44]. However, one must be cautious in striving for ever-increasing levels of sensitivity, since the most powerful PCR approaches are capable of detecting trace levels of *BCR-ABL1* transcripts even in healthy people who have never had CML [45].

The availability of such highly sensitive and quantitative methods has become even more important as we have entered the era of molecular targeted therapy for CML using specific inhibitors of the tyrosine kinase fusion protein (imatinib and its successors). While the vast majority of patients initially respond to these drugs, many later develop resistance, as evidenced by the creeping up of *BCR-ABL1* RT-PCR levels. (Very few treated patients, even if in apparent clinical and cytogenetic remission, actually go down to undetectable *BCR-ABL1* levels with these sensitive assays [46].) Most of these relapses are due to either amplification of the *BCR-ABL1* fusion gene or, more commonly, the development of point mutations affecting amino acid residues at the site of binding of the drug or at more distal sites causing allosteric effects. A total of about 20 such mutations have been described [47]. These can be detected by DNA sequencing or, as we have used, DNA microarray hybridization. Since certain mutations may render either similar

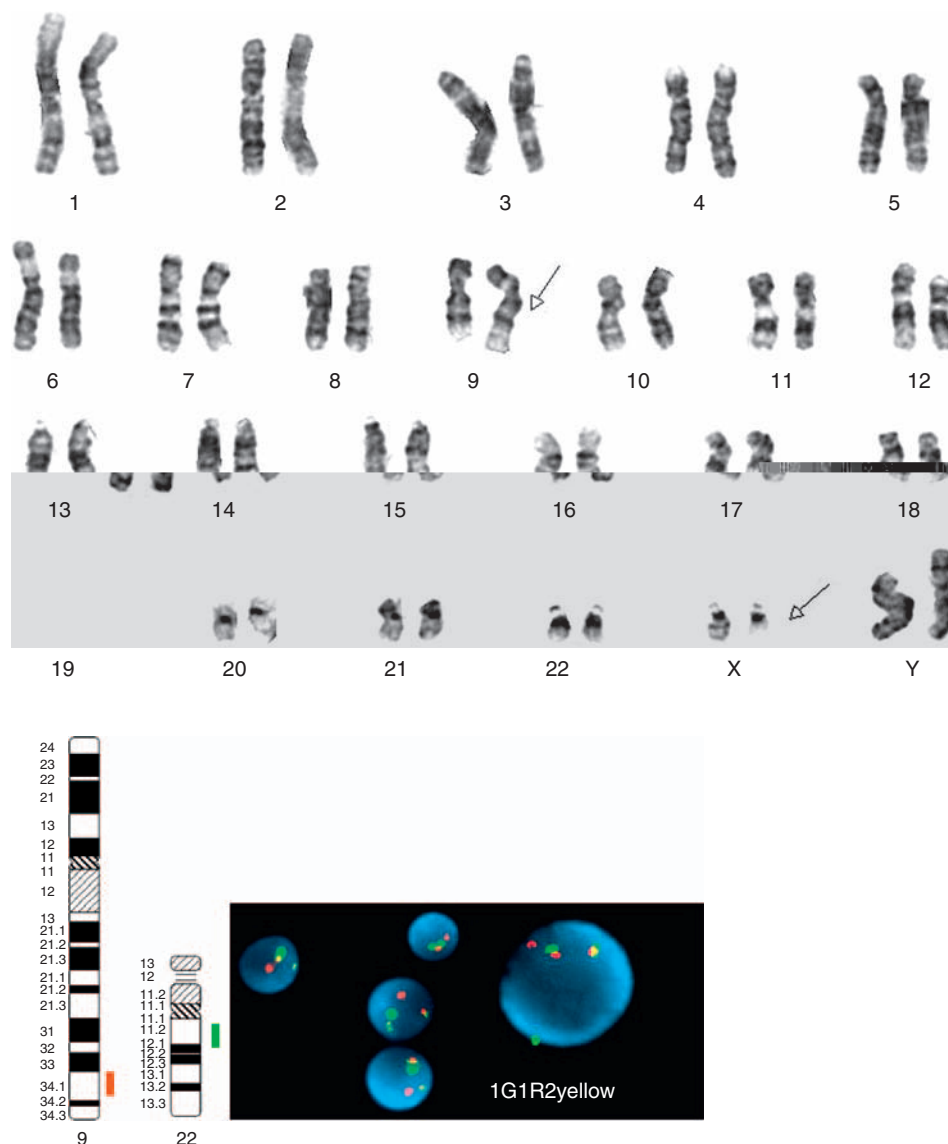


FIGURE 9.13 A G-banded karyotype with a classic t(9;22) showing the “Philadelphia” chromosome. An ideogram of chromosomes 9 and 22 showing the FISH probes for the *ABL* and *BCR* loci. The FISH panel shows cells with red *ABL*; green *BCR*, and yellow (Fusion) signals.

resistance or sensitivity to newer-generation drugs targeting the same protein, it will be important to accurately genotype these patients to guide management.

Cytogenetics

As described earlier, the *Ph* chromosome was the first consistent cytogenetic rearrangement found in a hematologic disease [48]. Banding techniques developed during the 1970s allowed for the identification of the *Ph* chromosome as being derived from a translocation between chromosomes 9 and 22, t(9;22)(q34;q11.2) (Figure 9.13) [49]. The translocation was subsequently described as resulting in the fusion of the *ABL1* protooncogene (a homolog of the Abelson murine leukemia virus oncogene) on chromosome 9q34 with a gene called *BCR* on chromosome 22q11.2 [50]. The *ABL1* gene encodes a tyrosine kinase that phosphorylates several proteins involved in signaling for cell proliferation, and the *BCR* gene encodes a

160-kDa phosphoprotein with kinase activity. The expression of the *BCR-ABL1* chimeric protein has an aberrant tyrosine kinase activity [51] and is leukemogenic [50–54].

The unambiguous presence of the *BCR-ABL1* fusion gene is required for a clinical diagnosis of CML and in typical cases remains the sole abnormality observed through most of the chronic phase. Approximately 90–95% of patients present with the t(9;22), whereas the remaining 5–10% of patients have cryptic or complex rearrangements but eventually fuse *BCR* and *ABL1*. Variant translocations with deletions at the involved breakpoints signify a poorer prognosis than the more common t(9;22) (Figure 9.14) [28, 29, 53]. FISH or molecular techniques can be used to establish diagnosis in cases where the t(9;22) cannot be identified by standard karyotyping.

CML-BC is often predicted by cytogenetic findings prior to pathologic changes; 75–80% of patients develop additional chromosome aberrations as the disease progresses.

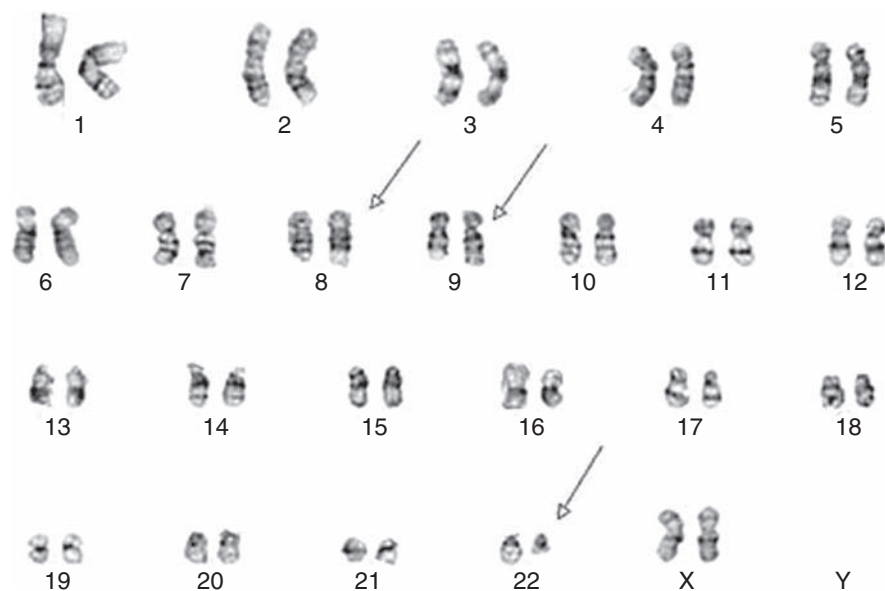


FIGURE 9.14 A G-banded karyotype showing a complex t(9;22;8) translocation with the “Ph” chromosome. Interphase FISH analyses on these cells (lower left) show one fusion signal (yellow) whereas the second fusion signal got rearranged on 9q and 8q subsequent to the initial 9;22 translocation. The lower right panel shows cells with a normal signal pattern (2 Red/2 Green) and a cell with a deletion of the reciprocal 22:9 fusion signal (1 Red/1 Green/1 Yellow).

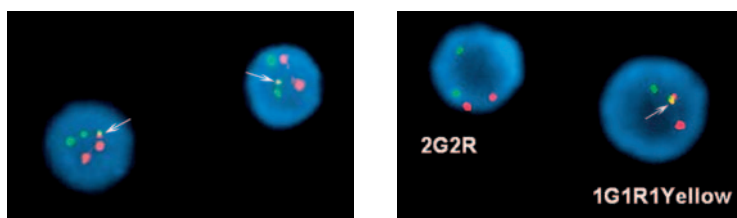
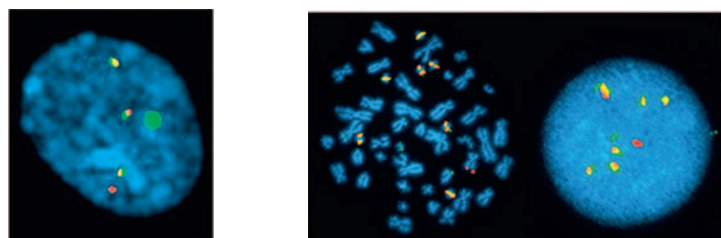


FIGURE 9.15 A G-banded karyotype of CML-BC, with an extra *Ph* chromosome, Trisomy 8, and isochromosome 17q. The FISH panel on the left shows three fusion signals (yellow) representing the additional *Ph* chromosome. The left panel shows the gains of additional “Ph” chromosome during development of imatinib resistance.



The aberrations of chromosomes 8, 17, 19, and 22 are most often involved in disease evolution (major route), accounting for approximately 70% of patients with evolving disease. Trisomy 8, isochromosome 17, trisomy 19, or

an extra *Ph* chromosome (derivative chromosome 22) is the most frequently observed secondary changes in blast crisis (Figure 9.15) [23, 55]. The remaining 30% of patients with evolving disease develop various secondary aberrations that

may include trisomy 21, loss of the Y, monosomy 7 or 17, trisomy 17, or others [55, 56]. Genes known to have roles in transformation include *TP53*, *RB1*, *CDKN2A*, *INK4α*, *MINK*, *AML1*, and *EVL1*, although their role in transformation is currently unknown [23, 57].

Clinical Aspects

CML represents between 15% and 20% of adult leukemias with a median age at diagnosis of about 50 years. The only known risk factor is exposure to ionizing radiation [24, 25]. The disease is asymptomatic in over 30% of the cases and is only suspected by the elevated WBC counts on routine blood examinations. Frequent clinical symptoms include fatigue, pallor, night sweats, and weight loss. Splenomegaly is observed in 50–75% of the patients. Occasionally, the disease presents at the blast phase without prior clinical manifestation of the chronic phase. The diagnosis is established by the demonstration of t(9;22)(q34;q11) (*Ph*¹) and/or *BCR-ABL1* fusion [49, 58, 59]. Transformation into accelerated or blast phase is usually associated with marked splenomegaly, severe anemia, and/or marked thrombocytopenia.

Molecular targeted therapy is now the recommended approach in the treatment of CML. The most exciting breakthrough in the treatment of CML has been the development of imatinib mesylate (IM, or Gleevec®) as an oral therapeutic agent. IM binds to a cleft between the N-terminal adenosine triphosphate binding domain and the C-terminal activation loop that forms the catalytic site of the Abl tyrosine kinase, locking the protein into the inactive conformation [54, 60]. Although IM appears to be extremely effective in CML, it has markedly reduced effectiveness in the acute leukemias. Patients with deletions at the *BCR-ABL1* breakpoint may not respond to therapy, and drug resistance can occur. IM resistance can occur via four main mechanisms. (1) The expression of the multidrug resistance P-glycoprotein increases drug efflux and decreases intracellular drug levels, thus decreasing drug effectiveness [61]. (2) The genomic amplification of the *BCR-ABL1* gene by gain of a second *Ph* chromosome or cellular aneuploidy is associated with resistance [62]. (3) The clonal evolution and development of chromosomal aberrations in addition to the t(9;22) may allow the clone to develop non-Bcr-Abl1-dependent growth mechanisms [63]. (4) Finally, *ABL1* gene mutations within the tyrosine kinase domain appear to prevent binding of IM to the protein [62].

Overall, the inhibitors of tyrosine kinase activity of Bcr-Abl have been more effective than the conventional drugs, such as interferon-alpha (IFN-α) combined with cytosine arabinoside (Ara-C). In the cases of no response or short-lived response to tyrosine kinase inhibitors, the possibility of bone marrow transplantation should be explored [64, 65].

CHRONIC NEUTROPHILIC LEUKEMIA

Chronic neutrophilic leukemia (CNL) is characterized by persistent peripheral blood neutrophilia, bone marrow hypercellularity, and hepatosplenomegaly [1, 66]. It is a rare

TABLE 9.4 WHO criteria for the diagnosis of chronic neutrophilic leukemia.*

1. Requirements
a. Peripheral blood
i. Persistent leukocytosis $\geq 25,000/\mu\text{L}$.
ii. Segmented neutrophils and bands $>80\%$ of the differential counts.
iii. Blasts $<1\%$ of the blood cells.
b. Bone marrow
i. Hypercellular with marked granulocytic preponderance.
ii. Myeloblasts $<5\%$ of the differential counts.
iii. No significant dysgranulopoiesis.
c. Hepatosplenomegaly
2. Exclusions
a. All causes of physiologic and reactive neutrophilia, such as infections, inflammations, tissue damage (infarctions, burns), or other malignancies.
b. All other chronic myeloproliferative diseases.
c. Myelodysplastic syndromes.
d. Myelodysplastic/myeloproliferative disorders.

*Adapted from Ref. [1].

condition which shares many morphological features with leukemoid reactions, but unlike leukemoid reactions, CNL is not associated with fever, infection, inflammatory process, or malignancy. In order to establish a diagnosis of CNL, all other myeloproliferative disorders and all causes of secondary (reactive) neutrophilia should be excluded (Table 9.4) [67, 68].

Etiology and Pathogenesis

The etiology and pathogenesis of CNL are not known. The frequent association of CNL with plasma cell myeloma in several reports may suggest release of cytokines by neoplastic plasma cells as the primary cause of neutrophilia [69]. However, clonality of CNL has been suggested by methylation studies of the X-linked hypoxanthine phosphoribosyl transferase gene and other probes [70]. Also, reports of the evolution of PV into CNL and the transformation of CNL to AML in certain cases support the clonal nature of this disorder [44]. Presence of the *JAK2* V617F tyrosine kinase mutation has been recently reported in CNL [72].

Pathology

Morphology

Bone marrow is hypercellular with marked granulocytic hyperplasia and an elevated M:E ratio approaching 10:1 or higher (Figure 9.16a). There is no evidence of increased blasts or promyelocytes. The erythroid line is unremarkable and megakaryocytes are either adequate or increased. No significant dysplastic changes are present. Bone marrow fibrosis is infrequent [1–4].

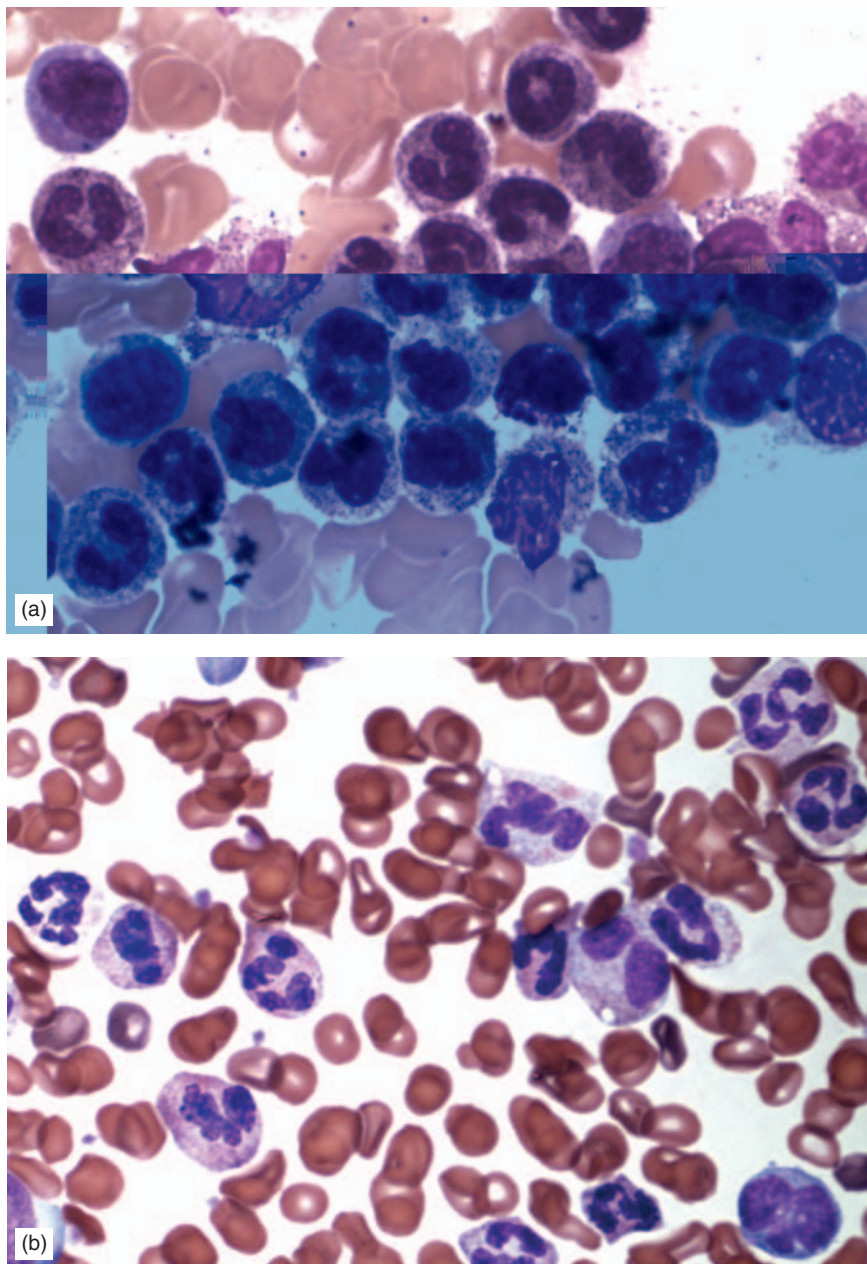


FIGURE 9.16 In chronic neutrophilic leukemia, bone marrow is hypercellular with marked preponderance of neutrophilic bands and segmented cells (a), and peripheral blood shows marked neutrophilia (b).

Blood smears show marked neutrophilia, usually $\geq 25,000/\mu\text{L}$, with a modest myeloid left shift and presence of scattered (5–10%) myelocytes and metamyelocytes (Figure 9.16b). Promyelocytes are rare and myeloblasts are commonly absent. Neutrophils may show toxic granulation. The LAP score is often elevated. Mild anemia and/or thrombocytopenia may be present [1–4].

Splenomegaly and hepatomegaly are due to neutrophilic infiltration in the splenic red pulp and hepatic sinusoids and/or portal areas [1].

Immunophenotypic Studies

The immunophenotypic characteristics of the bands and neutrophils in CNL are similar to those of normal neutrophils

and bands. So far, no aberrant expression or significant alteration of CD molecules have been reported.

Molecular Studies

No specific molecular markers exist for diagnosing CNL. Some cases show a *BCR-ABL1* fusion gene of the p230 isoform, detected as described earlier for CML (probably representing a variant of CML). Other cases may demonstrate the *JAK2* V617F mutation [71], described in detail later in this chapter.

Cytogenetic Results

No consistent chromosomal anomaly has been associated with CNL, and the primary genetic event is likely cryptic

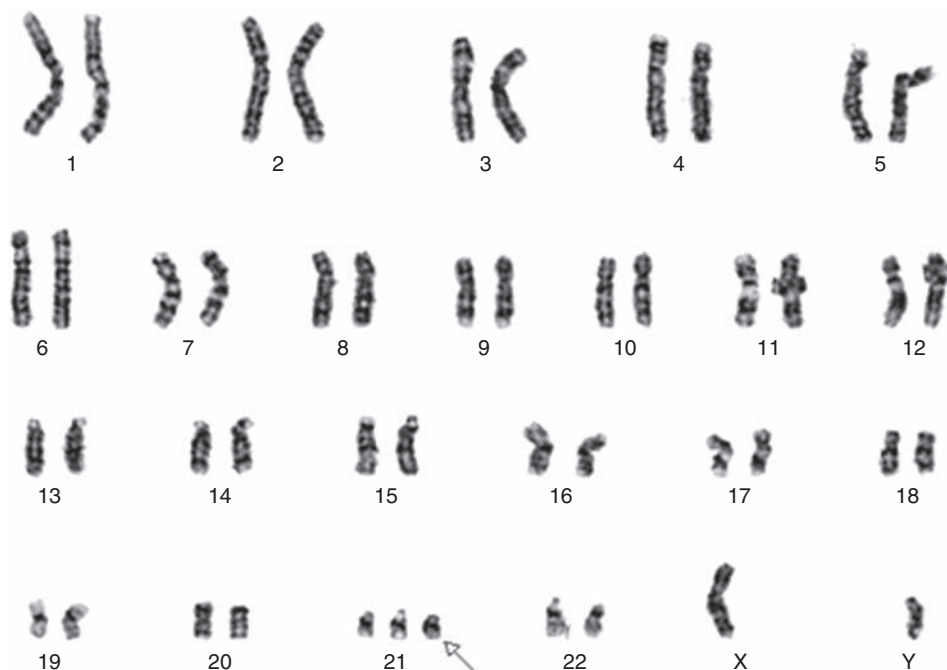


FIGURE 9.17 A G-banded karyotype with Trisomy 21.

(submicroscopic). Chromosomal anomalies reported to date may reflect secondary anomalies associated with chromosomal evolution in CNL. Sporadic reports of patients with +8, +9, del(20)(q11q13), del(11)(q14), +21 (Figure 9.17), and complex karyotypes are described in the literature [73, 74].

Clinical Aspects

CNL is a rare MPD which generally affects women and men over 60 years of age. In most cases, the disease behaves aggressively with a mean survival of <2 years [66, 73, 75]. The cause of death is often cerebral hemorrhage or infection. The transformation of CNL to AML has been reported [75]. There is also a report of CNL evolving from PV [76].

Due to the rarity of the disease, no standard therapeutic protocols are currently available. At the present time, allogeneic bone marrow transplantation appears to be the only potential cure. Certain drugs, such as hydroxyurea and IFN- α , may help to control granulocytosis and splenomegaly.

IDIOPATHIC HYPEREOSINOPHILIC SYNDROME AND CHRONIC EOSINOPHILIC LEUKEMIA

Idiopathic hypereosinophilic syndrome (HES) and chronic eosinophilic leukemia (CEL) represent overlapping persistent eosinophilic disorders with no known etiology, with a wide spectrum of clinical presentations ranging from indolent to aggressive clinical courses [1, 77–81]. HES is the preferred term when there is no evidence of clonality or increased blasts (Table 9.3). Neither HES nor CEL shows the *Pb1* chromosome or *BCR-ABL1* fusion gene.

Etiology and Pathogenesis

The etiology of HES and CEL is not known. The detection of *FIP1L1-PDGFR α* fusion gene in approximately 50% of the HES/CEL cases suggests a pathogenic role for tyrosine kinase activity of the fusion gene product in these disorders [82, 83]. The fusion of *FIP1L1* to *PDGFR α* is the result of a small interstitial deletion (only 800kb in size) of the long arm of chromosome 4, del(4)(q12q12) [84]. Also, an acquired t(8;9)(p21–23;p23–24) has been reported in some cases of CEL [77, 85, 86]. This translocation fuses the *PMC1* gene to the *JAK2* gene (a tyrosine kinase), further supporting the role of tyrosine kinase activity in the pathogenesis of chronic MPDs, including HES/CEL.

Pathology

Morphology

The bone marrow is hypercellular and shows eosinophilic hyperplasia. Eosinophils counts may range from 10% to 70% of the bone marrow nucleated cells, with a mean of about 30% [1, 87, 88]. The maturation of eosinophils and myeloid cells in many instances (HES) is progressive and orderly without significant left shift or increased blasts (Figure 9.18). But blasts are increased (>5% and <20%) in the smaller proportion of the cases (CEL) (Table 9.5). Charcot-Leyden crystals are frequent findings. Charcot-Leyden crystals are colorless, long hexagonal, double-pointed, or needle-like lysophospholipase containing structures formed from the breakdown of eosinophils. Eosinophils may show dysplastic changes such as nuclear hypersegmentation or hyposegmentation, cytoplasmic vacuolization or hypogranularity, and/or abnormal eosinophilic granules [1, 87, 88]. However, both abnormal morphologic changes and

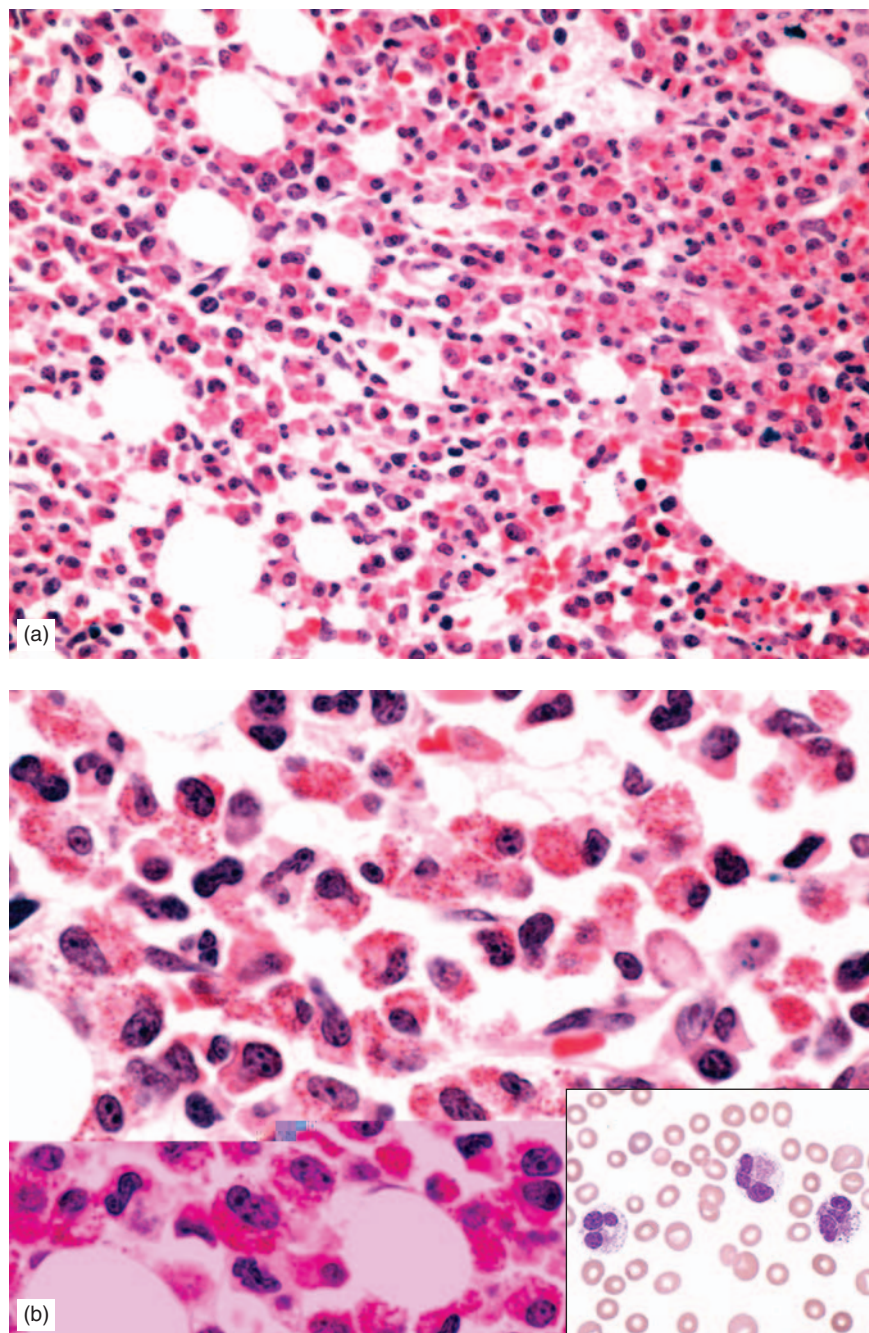


FIGURE 9.18 Bone marrow biopsy section (a and b) and blood smear (inset) of a patient with HES. Adapted from Naeim, F. (2001). *Atlas of Bone Marrow and Blood Pathology*, Saunders, by permission.

Charcot–Leyden crystals have been observed in cases of reactive eosinophilia. Myelofibrosis may be present but is not common.

The peripheral blood shows absolute eosinophilia ($>1,500/\mu\text{L}$) with or without neutrophilia, basophilia, myeloid left shift, or abnormal morphology. The leukocyte count is often moderately elevated (between 20,000 and 30,000/ μL), and eosinophils in most instances account for 30–70% of the differential counts [1, 87, 88].

Eosinophilic infiltration may also be present in the extramedullary sites. The site of infiltration usually shows some degree of fibrosis, often with the presence of Charcot–Leyden crystals.

Immunophenotypic Studies

The eosinophils show different characteristic features than the neutrophils by flow cytometry. They appear as distinct clusters in FSC/SSC and CD45/SSC dot plot analyses, and their intensity of expression of myeloid-associated markers is different from neutrophilic granulocytes. A number of CD molecules are expressed on eosinophils, such as CD9 (leukocyte antigen MIC3), CD32 (Fc γ RII), CDw125 (IL-5 receptor alpha chain), and CD193 (chemokine receptor 3), but these molecules are not eosinophilic-specific and are also expressed by other leukocytes [89, 90].

Anti-CD34 and -CD117 monoclonal antibodies can be used to estimate the number of blasts by flow cytometry or immunohistochemical stains in blood samples, bone marrow aspirates, or biopsy sections.

TABLE 9.5 WHO criteria for the diagnosis of chronic eosinophilic leukemia and hypereosinophilic syndrome.*

1. Requirements**

- Persistent peripheral blood eosinophilia $\geq 1,500/\mu\text{L}$.
- Bone marrow eosinophilia.
- Blasts $<20\%$ in blood or marrow.

2. Exclusions

- All causes of reactive eosinophilia secondary to allergic, parasitic, infections, pulmonary, and collagen vascular diseases.
- All neoplastic disorders with secondary, reactive eosinophilia, such as T-cell lymphoid malignancies, Hodgkin lymphoma, acute lymphoblastic leukemia/lymphoma, and mastocytosis.
- Neoplastic disorders that eosinophils are a part of the neoplastic clone, such as chronic myeloproliferative diseases, myelodysplastic syndromes, and acute myelogenous leukemia.
- Conditions associated aberrant expression or abnormal cytokine production of T lymphocytes.

*Adapted from Ref. [1].

**Diagnosis of chronic eosinophilic leukemia is made when items a–d are all excluded and if myeloid cells show clonal evolution by cytogenetic and/or molecular studies, or if the blast cells in the bone marrow are $>5\%$ and $<20\%$, or $>2\%$ blasts are present in the peripheral blood.

Molecular Studies

The only specific molecular finding in HES/CEL is the *FIP1L1-PDGFR* fusion gene, though it is only present in about half the cases [83, 84]. Since it is a tyrosine kinase, some patients have responded to imatinib therapy, so the finding has both therapeutic and diagnostic significance. And also like CML, resistance to the drug can arise from acquired mutations in the fusion gene, some of which may be sensitive to next-generation drugs [91].

Cytogenetics

Although no specific cytogenetic abnormalities have been associated with CEL, the presence of another clonal anomaly that is associated with MPD can help in the differential diagnosis between CEL and a reactive disease that involves the eosinophils. Cytogenetic anomalies in CEL often have been associated with a poor prognosis [92]. One important chromosomal aberration that has been linked with CEL is $t(5;12)$ (Figure 9.19). The $t(5;12)$ results in the fusion of the *PDGFR* β tyrosine kinase gene on chromosome 5q33 and the *TEL* gene on chromosome 12p12. Various investigators have assigned the breakpoints within chromosome 5 as 5q33 or 5q31, and within chromosome 12 as p12 or p13; however, the investigators are most likely describing the same translocation [13]. Variant translocations that involve the *PDGFR* gene include the $t(5;7)(q33;q11.2)$ and $t(5;10)(q33;21.2)$. Fusion of the *FIP1L1* gene to the platelet-derived growth factor receptor alpha (*PDGFR* α) gene has recently been described in patients with HES [93]. These two genes lie very close to one another within chromosome band 4q12, so the fusion cannot be detected by karyotype. However,

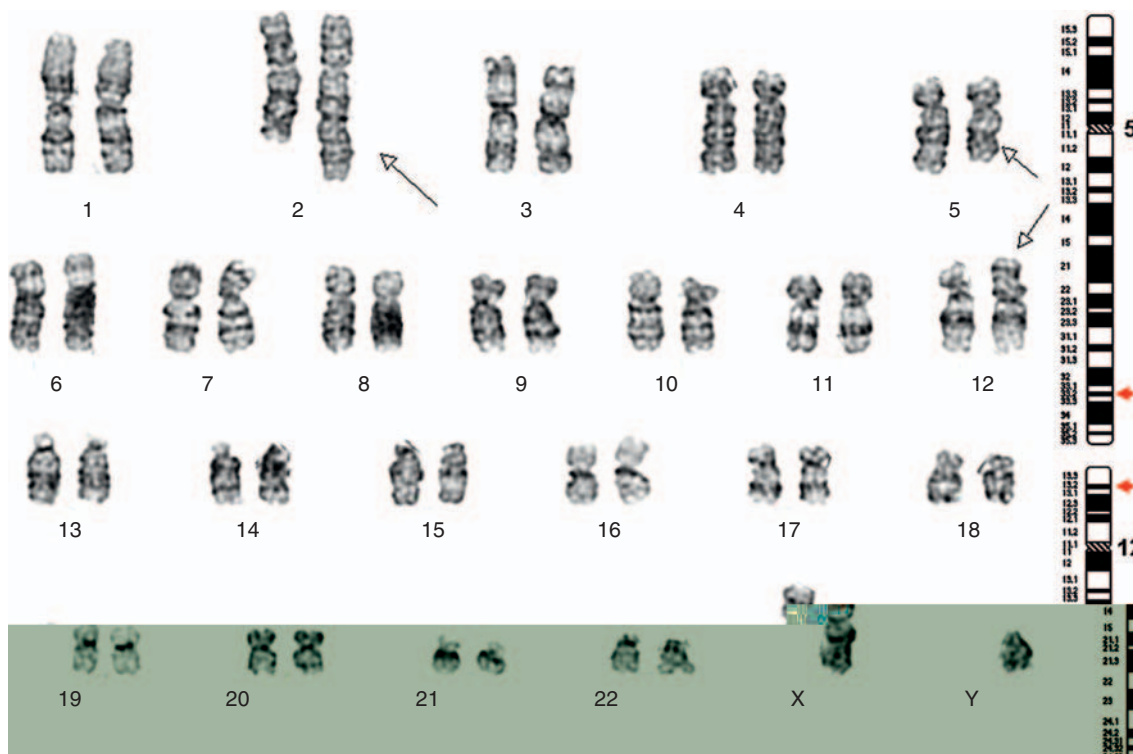


FIGURE 9.19 A G-banded karyotype with a balanced 5;12 translocation. (The 2q abnormality is a secondary event.)

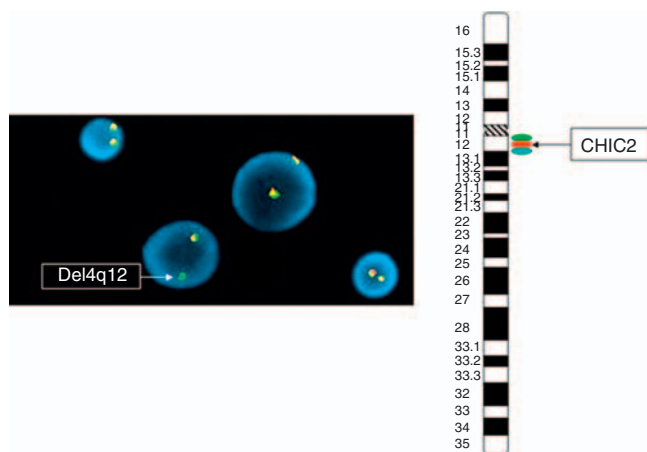


FIGURE 9.20 Deletion of the 4q12 region as identified by 3-color FISH.

the fusion results in a deletion of the intervening DNA sequences (CHIC2 deletion) (Figure 9.20), which can be detected *only* by FISH and is a target for IM treatment [93].

Clinical Aspects

HES and CEL involve men much more frequently than women (M:F ratio about 9:1). They are usually detected between the ages of 20 and 50 years and are rare in children. The most common clinical symptoms include fatigue, cough, dyspnea, myalgia, angioderma, rash, fever, and rhinitis [77–81]. The release of eosinophilic granules may damage the endocardium and the endothelial cells and lead to thrombus formation and emboli. Endocardial thrombosis and fibrosis may cause insufficiencies of the mitral or tricuspid valves [94].

The presence of blast cells in the peripheral blood, increased blasts in the bone marrow, multilineage dysplasia, marked splenomegaly, and cytogenetic aberrations are considered signs of adverse clinical outcome.

POLYCYTHEMIA VERA

Polycythemia vera (PV) is characterized by erythrocytosis or increased red blood cell mass with hyperviscosity, increased risk of thrombosis, and varying degrees of thrombocytosis, leukocytosis, and splenomegaly [1, 95]. PV, similar to the other myeloproliferative diseases, is the result of clonal expansion of a pluripotent stem cell with the involvement of the myeloid lineages and a variable proportion of B lymphocytes. However, the majority of the T and NK cells do not seem to be affected.

The diagnosis of PV is established by complex clinical, laboratory, and morphologic features. The recommended criteria for the diagnosis of PV by WHO consist of two major categories [1].

Category A

1. Elevated RBC mass >25% above normal range, of Hb >18.5 g/dL in men and >16.5 g/dL in women.

2. No evidence of familial erythrocytosis or elevated erythropoietin (Epo) due to:
 - a. Hypoxia (arterial $pO_2 \leq 92\%$)
 - b. High-oxygen-affinity hemoglobin
 - c. Truncated Epo receptor
 - d. Inappropriate Epo production by tumors.
3. Splenomegaly.
4. Clonal genetic abnormalities other than *Pb1* or *BCR-ABL1* fusion gene.
5. Endogenous (erythropoietic-independent) erythroid colony formation *in vitro*.

Category B

1. Thrombocytosis >400,000/ μ L.
2. WBC >12,000/ μ L.
3. Bone marrow demonstrating panmyelosis with erythroid preponderance and megakaryocytosis.
4. Low serum erythropoietic levels.

The diagnosis of PV is made when A1 and A2 plus any other category A are present or when A1 and A2 plus any two of category B are present.

Etiology and Pathogenesis

The etiology of PV is not known. A genetic predisposition has been suggested based on the reports of PV in identical twins [96]. Also, a higher incidence of PV has been observed in Hiroshima atomic bomb survivors, US military personnel involved in the nuclear weapons tests, and persons with occupational exposure to chemical toxins.

The erythroid precursors seem to be erythropoietin (Epo) independent in tissue culture settings. The presence of erythroid colonies in the absence of exogenous Epo is considered an *in vitro* PV hallmark. The PV erythroid progenitors are also hypersensitive to Epo as well as to other hematopoietic growth factors and differentiate faster than their normal counterparts. In addition, it has been shown that the BFU-E cells from PV patients have increased sensitivity to insulin-like growth factor-1 (IGF-1). IGF-1 has an Epo-like activity and stimulates erythropoiesis [14].

One of the interesting current hypotheses in the pathogenesis of PV is the presence of a defect in transcription regulation that affects cytokine receptor signaling. It has been shown that the *JAK2* gene plays an important role in the EOP-EPO receptor signaling in erythropoiesis. *JAK2* mutation in mice during embryogenesis is lethal due to the lack of sufficient erythropoiesis. A unique clonal mutation in the *JAK2* gene has been reported, resulting in a valine to phenylalanine substitution at position 617, in 65–97% of PV patients [14].

Pathology

Morphology

Two distinct clinopathologic phases have been described in PV: the polycythemic phase and the “spent” phase [1–4,

95, 97]. Polycythemic phase describes the earlier active phase of the disease when the bone marrow shows panmyelosis and blood displays erythrocytosis, sometimes in association with thrombocytosis and/or leukocytosis. Spent phase refers to the later stage of the disease when bone marrow is fibrotic or hypocellular, and there is evidence of extramedullary hematopoiesis, progressive splenomegaly, and anemia. In addition, a *transitional phase* has been described, referring to a process between these two phases characterized by erythrocytosis and bone marrow fibrosis. The morphologic features of the polycythemic and spent phases are described below.

Polycythemic phase is characterized by bone marrow hypercellularity and panmyelosis. Usually, there is marked erythroid preponderance and predominance of megakaryocytes (Figure 9.21). Erythropoiesis is normoblastic, and megakaryocytes are pleomorphic and have a tendency to appear in clusters and/or next to bone trabeculae. No significant dysplastic changes are noted in the granulocytic series and there is no evidence of increased myeloblasts. Basophilia is a common feature, and eosinophilia is not infrequent. In the majority of PV cases at this phase, bone marrow biopsies show no increase in reticulin fibers, though variable degrees of fibrosis are present in about 30% of the cases. Stainable iron is reduced or absent in the vast majority of cases [1–4, 95, 97].

The peripheral blood shows increased red cell mass and elevated hemoglobin levels. Erythrocytes are usually normochromic and normocytic, but sometimes are hypochromic and microcytic. The presence of deeply basophilic reticulocytes has been described. The activity of red cell glycolytic enzymes and the proportion of fetal hemoglobin are increased. There is often leukocytosis and thrombocytosis with basophilia and presence of giant, hypogranular platelets. There may be mild myeloid left shift but blasts are not usually found. The LAP score is often elevated.

“Spent” phase represents the late stage of PV and often is characterized by [1–4, 95, 97]:

1. Normalization of erythrocytosis and then progression to anemia.
2. Progressive splenomegaly.
3. Myelofibrosis and extramedullary hematopoiesis with leukoerythroblastic blood picture.
4. Myelodysplastic changes, sometimes with increased blasts.

There is some debate regarding the mechanism(s) involved in the evolution of PV to the spent phase. The original idea of the development of anemia in PV as the natural history of the disease due to bone marrow exhaustion has been challenged by the causative effects of chemotherapy, hemorrhage, and deficiencies of iron, vitamin B₁₂, or folic acid. Similarly, the development of myelodysplasia and/or marrow fibrosis may be more secondary to therapy than a naturally occurring phenomenon. For example, the incidence of myelofibrosis is higher in PV patients exposed to chemotherapy or radiation than those treated by phlebotomy.

Immunophenotypic Studies

No pathognomonic immunophenotypic features have been described in the blood or bone marrow of PV patients.

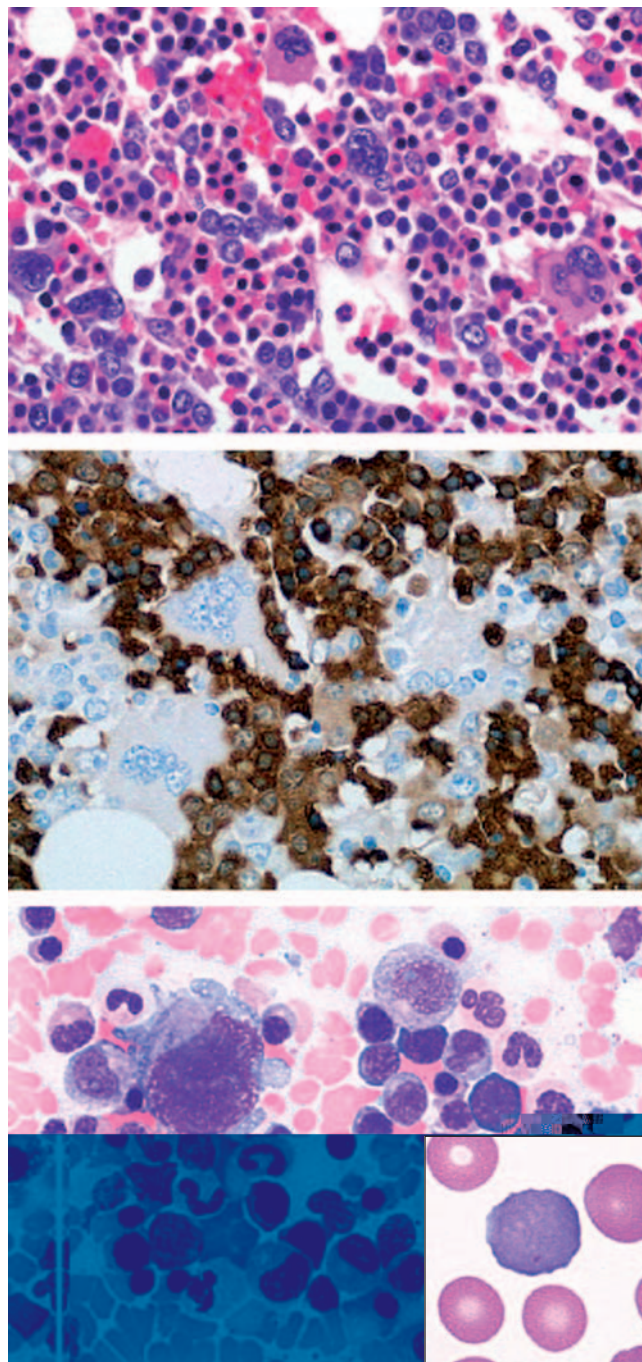


FIGURE 9.21 Bone marrow biopsy section (top) and smear (bottom) from a patient with PV demonstrating marked erythroid preponderance. The figure in the middle depicts hemoglobin A by immunohistochemical stains. The inset represents a deeply stained polychromatophilic erythrocyte.

Molecular Studies

A specific molecular marker for a number of MPD is a point mutation in the *JAK2* gene, V617F. Within this group, it is most frequently seen in PV, where some series find it in >90% of affected patients [98–100]. As such, it can be of help in the diagnosis of PV, and in its differential diagnosis from other disorders such as CML, chronic myelomonocytic leukemia, acute leukemia, and MDS. Since

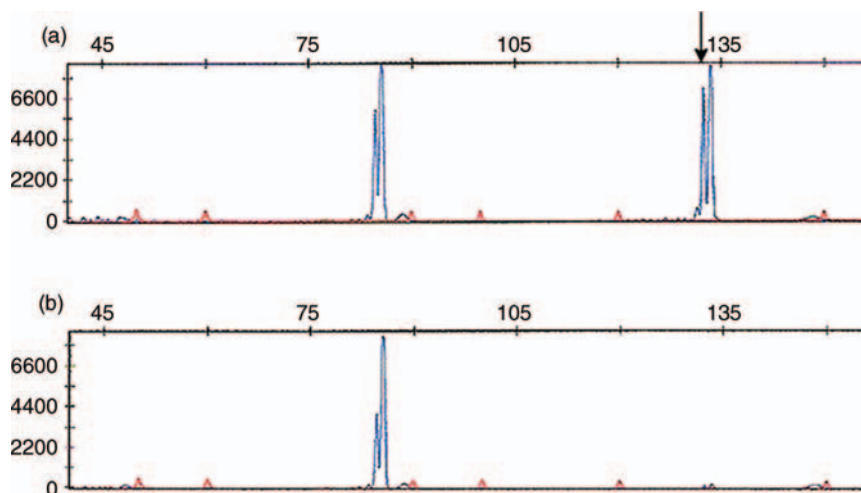


FIGURE 9.22 Detection of the *JAK2* mutation by allele-specific PCR followed by capillary electrophoresis of the amplification products. A positive sample (a) yields both the mutant PCR product (arrow) and the internal control normal gene product, whereas a negative sample (b) yields only the normal PCR product peak.

it is a single nucleotide change, any of a variety of straightforward assays can be used. Allele-specific PCR primers can be used to amplify both the mutant region of the gene (if present) and a nearby invariable portion of the gene (as an internal amplification control). The method also allows for semi-quantitative assessment of the proportion of *JAK2* mutant cells present, by comparing the heights of the two PCR peaks on capillary electrophoresis, which may have prognostic significance (Figure 9.22). Other methods in use include real-time PCR incorporating melting-curve analysis [99] and DNA sequencing or pyrosequencing [100]. Most of these methods are not very good at distinguishing homozygous from heterozygous *JAK2* mutations, which may also have some influence on clinical behavior. Homozygosity results from mitotic recombination in the *JAK2*-positive cells and/or loss-of-heterozygosity at the gene locus on chromosome 9p [98].

Another potential marker is the *MPL* gene for the thrombopoietin (TPO) receptor which shows reduced expression in platelets and megakaryocytes in PV [101].

Cytogenetics

In PV, cytogenetic results do not predict evolution of the disease, but they can provide clues to hematologic phenotype, duration of the disease, and consequences of myelosuppressive therapy [15]. A greater proportion of patients with advanced disease (and poorer prognosis) have chromosomally abnormal clones than patients with early stage PV [11, 15]. In addition, abnormal clones are more frequent among patients who have PV with myeloid metaplasia (78%) than among patients who have PV alone (19%) or PV with myelofibrosis (40%) [15]. The most common chromosomal abnormalities at PV diagnosis are $\text{del}(20)(\text{q}11\text{q}13)$, $+8$, and $+9$, with $+8$ and $+9$ often occurring together in the same clone. Additional abnormalities observed include $\text{del}(1)(\text{p}11)$, $\text{del}(3)(\text{p}11\text{p}14)$, $\text{t}(1;6)(\text{q}11;\text{p}21)$, and $\text{t}(1;7)(\text{q}10;\text{p}10)$ (Figure 9.23). In some patients with PV, a *de novo* leukemia or MDS develops; in these patients, chromosomal anomalies are more similar to the secondary disease than those associated with untreated PV. Still other patients with PV develop a chromosomally abnormal clone as a

consequence of therapy. The most common chromosomal anomaly associated with therapy-related leukemia involves anomalies of chromosome 5 or 7 or both and unbalanced translocations derived from $\text{t}(1;7)(\text{q}10;\text{p}10)$ [16].

Several studies have shown that an abnormal karyotype at diagnosis of PV is associated with a poor prognosis, while the proportion of patients with an abnormal karyotype increases during the course of the disease [15, 102, 103]. PV may progress to a terminal phase, which can involve transformation to myelofibrosis or acute leukemia. Almost all the PV patients who develop acute leukemia in late disease stages have chromosomal abnormalities. Trisomy 8 or 9 may persist in PV without further clonal evolution or leukemia development for up to 15 years, whereas other chromosomal abnormalities, such as -7 or $5\text{q}-$ or complex changes, may signal the terminal phase of the disease. The utilization of FISH methods in PV did not detect a substantially increased incidence rate of the chromosomal abnormalities, or did it reveal submicroscopic deletions in patients with a normal karyotype. However, both FISH and comparative genomic hybridization studies have revealed frequent abnormalities of chromosome 9, including gains in 9p.

Clinical Aspects

PV is more frequent in men than in women (about 2:1) with a peak incidence around 70–80 years of age. Most common complaints are non-specific and include headache, weakness, and dizziness. Approximately 5–20% of the patients may complain of arthritis. Itching, particularly after a warm bath, and erythromelalgia (erythroderma with burning pain of extremities) are common features. Although the reports of survival time for untreated patients range from 6 to 18 months, treated patients live much longer, usually exceeding 10 years. The major causes of death are thrombosis, transformation to MDS or AML, non-hematologic malignancies, and hemorrhage [104–107].

Thrombosis is either venous or arterial and appears to be primarily related to the patient's blood hyperviscosity. Transformation to MDS or AML in most instances is related

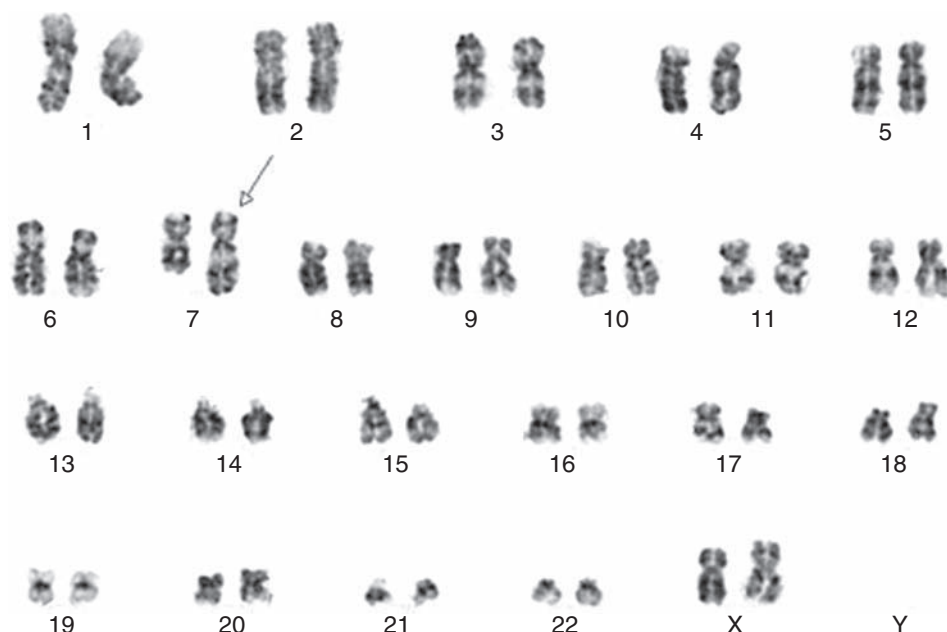


FIGURE 9.23 A G-banded unbalanced 1;7 translocation, resulting in three copies of 1q and loss of one copy of 7q.

to chemotherapy. In one report the incidence of AML in PV patients was 1.5%, 10%, and 13% for phlebotomy, ^{32}P , and chlorambucil therapy, respectively [105–107]. Approximately 3–12% of the patients may develop a secondary malignancy (carcinoma, non-Hodgkin lymphoma). The risk of secondary malignancies is much higher in patients who receive myelosuppressive therapy. Because of the high risk of development of malignancies, chlorambucil therapy has been discontinued. Other therapeutic modalities include IFN- α , anagrelide (a quinazoline derivative), and allopurinol [105–107].

CHRONIC IDIOPATHIC MYELOFIBROSIS

Chronic idiopathic myelofibrosis (CIMF) or agnogenic myeloid metaplasia or myelofibrosis with myeloid metaplasia is a clonal stem cell disorder and a subtype of CMPD characterized by myeloproliferation, atypical megakaryocytic hyperplasia, bone marrow fibrosis, extramedullary hematopoiesis, and marked splenomegaly [1, 108–110]. These changes often lead to anemia, leukoerythroblastosis (the presence of immature myeloid and erythroid cells in blood), and tear-drop-shaped red cells.

Etiology and Pathogenesis

The etiology of CIMF is not known. However, in a small proportion of the cases, development of CIMF has been linked to ionizing radiation, thorium dioxide, and petroleum derivatives, such as toluene and benzene [109]. Also, a mutation of the *GATA-1* transcription factor gene in mice may induce pictures similar to those of myelofibrosis.

Studies based on X-chromosome genes, karyotyping, or identification of *RAS* and *JAK2* gene mutations,

are consistent with a clonal stem cell involvement [111]. Cytogenetic aberrations with chromosomal deletions, such as 13q $^{-}$, 20q $^{-}$, and 12p $^{-}$, have been frequently reported in CIMF, as well as trisomies 8 and 9 [112]. Pathologic features consist of two fundamental components: myelofibrosis and extramedullary hematopoiesis.

Myelofibrosis appears to be a reactive process secondary to the activation of bone marrow stromal cells, particularly fibroblasts. Bone marrow fibroblasts in CIMF are polyclonal and structurally normal. Pathogenesis of myelofibrosis, increased number of stromal cells, and excess deposition of extracellular matrix proteins is probably mediated by the release of regulatory cytokines secondary to the clonal proliferation of the hematopoietic cells [111–113]. For example, transforming growth factor-beta (TGF- β) is a glycoprotein that is produced and released by endothelial cells, monocytes, and megakaryocytes. TGF- β enhances the production and release of extracellular matrix protein, such as collagen from fibroblasts [108–111]. A CIMF-like condition has been induced in mice by administration of high dose TPO. TPO induces megakaryocytosis and, therefore, increased release of TGF- β , resulting in bone marrow fibrosis [113–115].

Another possible mechanism for the induction of myelofibrosis in CIMF is increased rate of emperipoiesis (entry of hematopoietic cells into megakaryocytic cytoplasm) observed by some investigators in experimental animal models [116]. Emperipoiesis, which is probably induced by abnormal localization of P-selectin on the megakaryocytes, entraps the hematopoietic cells, causes cell damage, and releases growth factors leading to the activation of fibroblasts and fibrosis.

Extramedullary hematopoiesis is probably due to the abnormal release of hematopoietic precursors from bone marrow sinusoids into the circulation and their homing in other tissues, particularly spleen and liver. Bone marrow

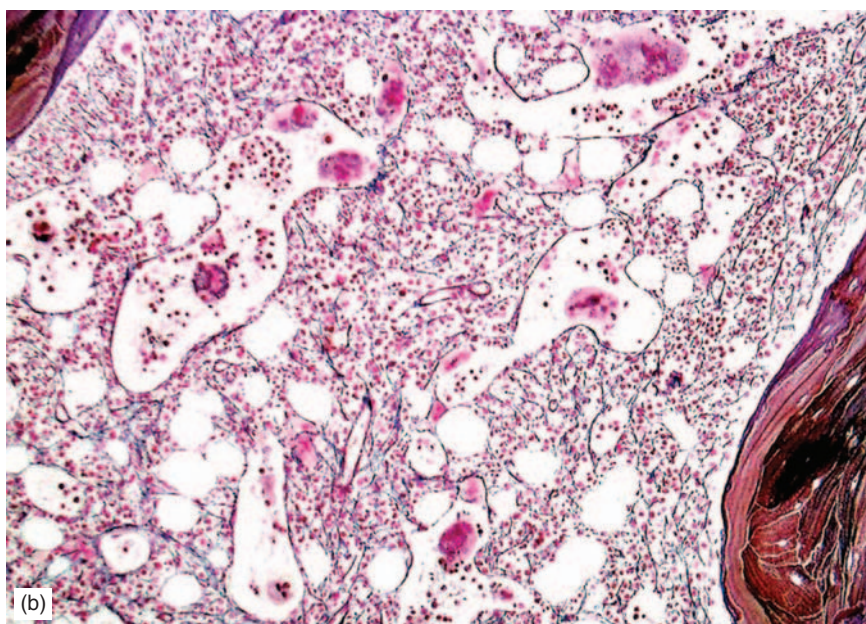
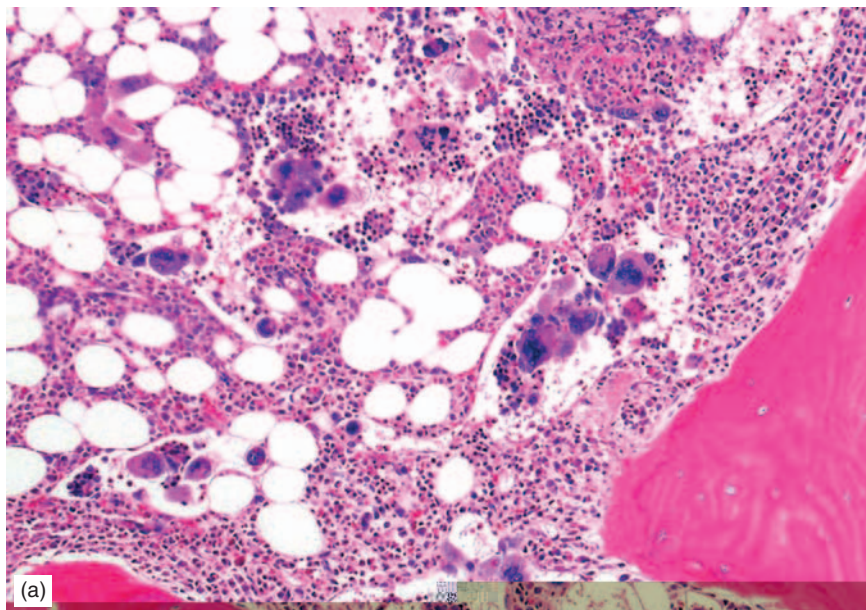


FIGURE 9.24 Biopsy sections in cellular phase of CIMF often show dilated hematopoietic-containing sinuses: (a) minimal amount of increased reticulin fibers and (b) reticulin stain.

fibrosis distorts the normal structure of the sinusoids, causing collapse of some and dilatation of others. These changes may disrupt the gatekeeping role of the sinusoids and cause the release of immature cells. In addition, the dilated sinusoids often show aggregates of hematopoietic precursors that could be released into the circulation.

Pathology

Morphology

The morphologic features of CIMF, such as bone marrow cellularity, extent of fibrosis, peripheral blood findings, and extramedullary hematopoiesis, vary considerably in different stages of the disease. The evolutionary process of CIMF

in bone marrow and blood can be divided into two major phases: cellular and fibrotic [1–4, 117].

Prefibrotic stage or **cellular phase** represents the early stage of the disease, when bone marrow fibrosis is lacking or there is only a minimal amount of reticulin fibrosis (Figure 9.24). At this stage, the bone marrow, similar to the other chronic MPDs, is hypercellular and displays panmyelosis. Megakaryocytes are atypical and often appear in clusters around the sinusoids and/or bone trabeculae (Figure 9.25). Abnormal nuclear lobulation, naked nuclei, and large bizarre forms are frequent findings. Micromegakaryocytes are often present. There may be a myeloid left shift with a higher proportion of the intermediate cells and <10% myeloblasts. Lymphoid aggregates are present in up to 25% of the cases.

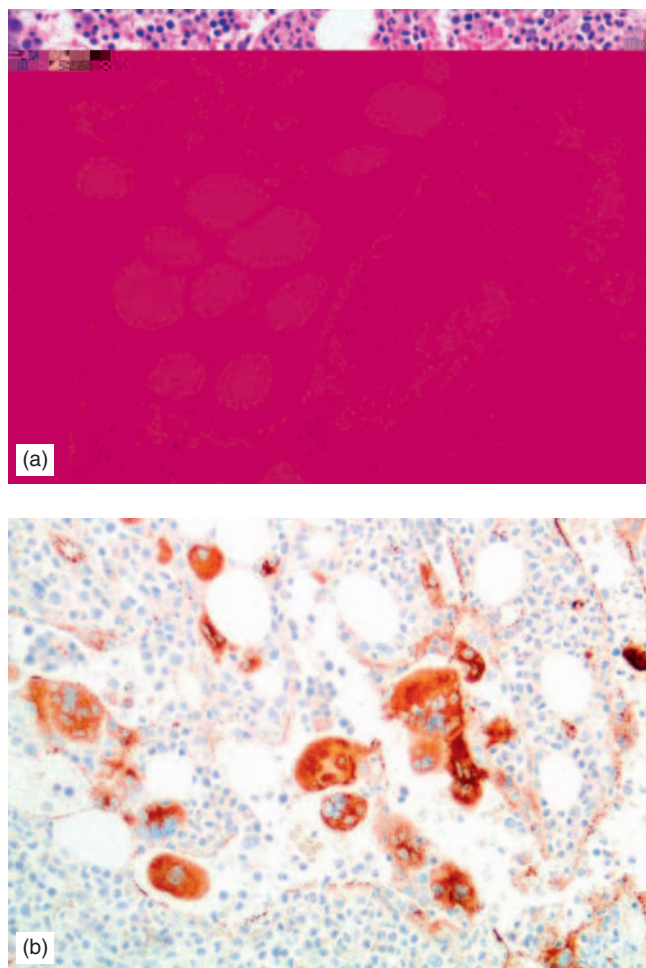


FIGURE 9.25 Biopsy section of cellular phase of CIMF demonstrating a dilated sinus containing numerous megakaryocytes and erythroid and myeloid precursors (a). Numerous megakaryocytes are demonstrated by immunohistochemical stain for factor VIII (b).

Leukocytosis, thrombocytosis, and mild to moderate anemia are the frequent peripheral blood findings. Anisopoikilocytosis is mild and occasional tear-drop-shaped red blood cells may be present. Leukoerythroblastosis is either mild or lacking. Giant platelets may be present.

Establishment of the diagnosis of CIMF at this stage is often difficult because of significant overlapping features with other chronic MPD, particularly idiopathic thrombocytopenia. Their differential diagnoses are discussed at the end of this chapter.

Fibrotic stage represents the more advanced phase of the disease characterized by various degrees of fibrosis and reduced bone marrow cellularity (Figures 9.26 and 9.27). The extracellular matrix consists of excessive amounts of type I, III, IV, and V collagen, reticulin (glycoprotein coating of stromal cell strands), fibronectin, and laminin. Reticulin and type III collagen are the predominant components. Reticulin precedes the excessive collagen deposits and stains black by silver impregnation. Collagen appears bluish-green by trichrome stain and is usually detected in advanced fibrosis.

Osteosclerosis and dilatation of bone marrow sinusoids are common features. The dilated sinusoids often contain aggregates of hematopoietic precursors, including dysplastic megakaryocytes. The bone marrow in advanced stages of the disease is virtually replaced by a dense fibrous tissue with markedly reduced cellularity and scattered trapped dysplastic megakaryocytes and small islands of erythroid and myeloid precursors. The bone marrow aspiration is usually unsuccessful and results in a “dry” tap.

Blood examination reveals leukoerythroblastic morphology with the presence of immature myeloid and erythroid cells (Figure 9.28). Blasts may be present but are usually <5%. Tear-drop-shaped red cells (dacryocytes) are commonly present. Anemia is a frequent finding, and there is often mild to moderate leukocytosis. Hypersegmented neutrophils may be present. The platelet count may be increased or reduced with abnormal forms present. Bare megakaryocytic nuclei and micromegakaryocytes are often detected. Significant dysplastic changes in myeloid series and blasts >10% are suggestive of an AP. The LAP score is often increased, and serum levels of lactate dehydrogenase and uric acid may be elevated.

Extramedullary hematopoiesis is often observed in the spleen and liver, but is also seen in other sites, such as lymph nodes, lung, serosal surfaces, urogenital system, skin, and retroperitoneal and paraspinal spaces (Figure 9.29). In the spleen, the red pulp is involved with the presence of erythroid, myeloid, and megakaryocytic cells in the sinuses. The extent of red pulp involvement and the proportion of each hematopoietic lineage vary from case to case, but megakaryocytes are commonly prominent. Similarly, sinuses are the main sites of extramedullary hematopoiesis in the liver and the lymph nodes. The splenic cords and the hepatic parenchyma may show various degrees of fibrosis.

Immunophenotypic Studies

No pathognomonic immunophenotypic features have been described in the blood, bone marrow, or other tissues in patients with CIMF.

Molecular and Cytogenetic Studies

There are numerous reports regarding the association between *JAK2* mutation (*JAK2*^{V617F}) and *BCR-ABL*-negative CMPD [118]. In a large study of 157 patients with myelofibrosis with myeloid metaplasia, the rate of *JAK2* mutation was about 45% [118].

Although the proportion of cases of CIMF with abnormal karyotypes ranges from 30% to 75%, distinct recurrent chromosomal aberrations have been reported in 40–50% of patients. This discrepancy is mostly due to difficulty in sampling adequate numbers of quality metaphases from the few cells aspirated from fibrotic marrow [20, 119, 128]. Although no “specific” chromosome anomalies are observed in patients with CIMF, +1q, del(13q), del(20q), and +8 appear in approximately two-thirds of patients with pathologic karyotypes [20], and rarer anomalies include +9 and del(12p). The most common anomalies (del(13q) and translocations involving chromosome 13q14) likely interrupt the *RB1* gene, an important tumor-suppressor gene in retinoblastoma, osteosarcoma, and other solid tumors [15]. Although balanced

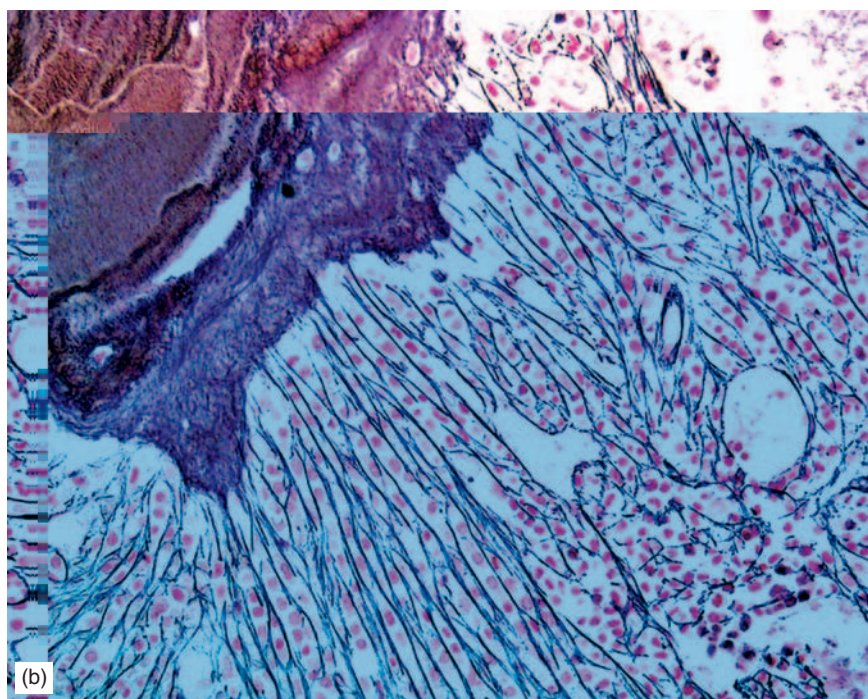
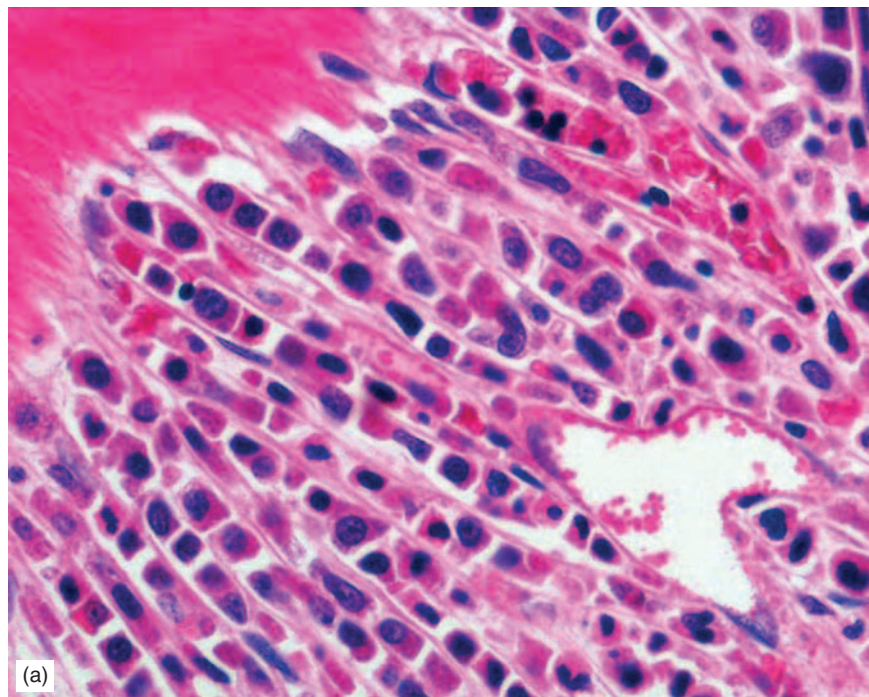


FIGURE 9.26 Biopsy section of a case of CIMF demonstrating separation of hematopoietic cells by delicate fibers (a) which are positive with reticulin stain (b).

translocations are uncommon, some reports document isolated cases with balanced translocations mostly involving chromosomes 1 and 12 with different partners [15, 119]. Specific cytogenetic abnormalities in CIMF are associated with significantly different survival outcomes [120, 121]. Prognostically favorable aberrations include 13q⁻ and 20q⁻, whereas prognostically unfavorable clones may contain 12p⁻ and +8 [122].

Clinical Aspects

The incidence of CIMF is approximately 1 per 100,000 with a median age of about 65 years. Men and women are equally affected. Marked splenomegaly is the hallmark, particularly in advanced stages. Non-specific symptoms such as fatigue, weight loss, night sweats, and fever are often present. Patients

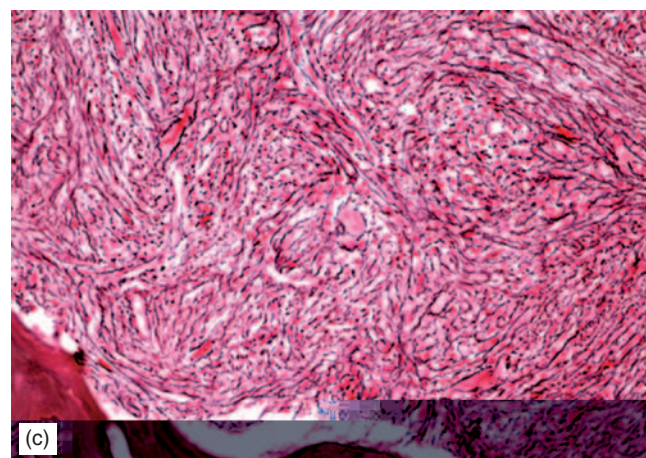
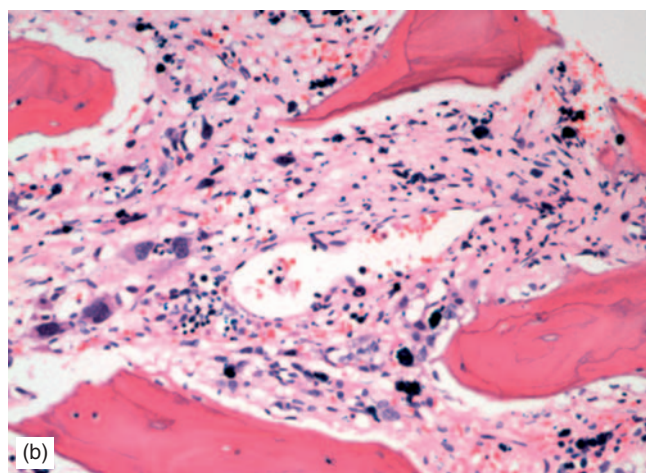
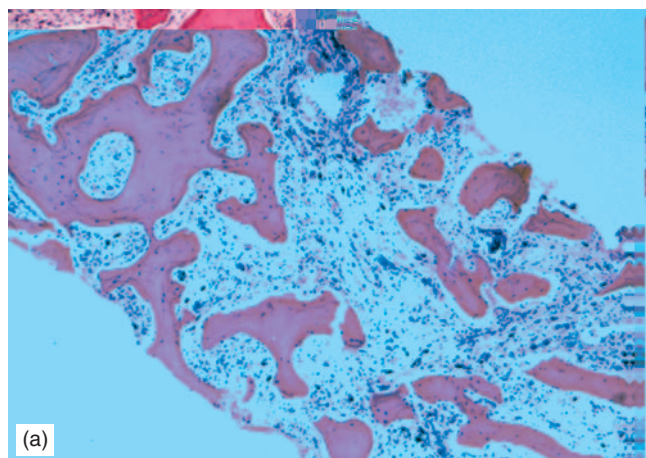


FIGURE 9.27 The advanced (fibrotic) stage of CIMF is characterized by extensive fibrosis and reduced number of hematopoietic cells: (a) low power, (b) high power, and (c) reticulin stain.

frequently show anemia with abnormal (low or high) white cell and/or platelet counts. Serum lactate dehydrogenase levels are elevated [108–110].

Marked splenomegaly may lead to splenic infarction, portal hypertension, and thrombosis of the small portal veins. Advanced age, anemia, and chromosomal abnormalities

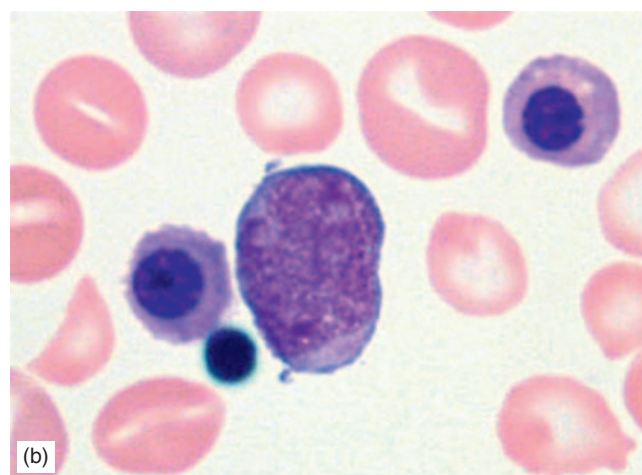
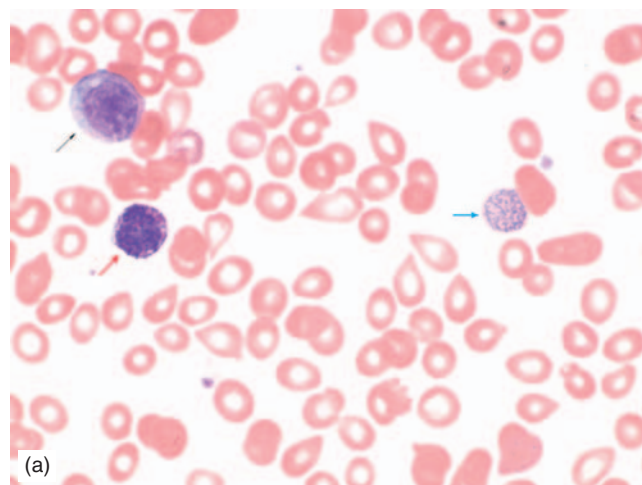


FIGURE 9.28 Peripheral blood smears from a patient with CIMF. (a) Low power demonstrating numerous tear-drop-shaped erythrocytes, basophilic stippling (blue arrow), a myelocyte (black arrow), and a basophil (red arrow). (b) High power showing two nucleated red cells and a myeloblast (leukoerythroblastosis).

are considered poor prognostic indicators. Anemia in about 20% of the patients is severe ($\text{Hb} < 8 \text{ g/dL}$) and may be due to several factors, such as reduced bone marrow erythropoietic sites, ineffective erythropoiesis, splenic sequestration and destruction of red cells, autoimmune hemolysis, and/or bleeding [114].

Therapeutic approaches include treatment with hydroxyurea, splenectomy, splenic irradiation, allogeneic or autologous stem cell transplantation, and the use of antian-giogenic drugs [110].

ESSENTIAL THROMBOCYTHEMIA

Essential thrombocythemia (ET), or primary thrombocytosis, is a clonal stem cell disorder and a subtype of CMPD characterized by protracted thrombocytosis in the peripheral blood and increased number of megakaryocytes with atypical features in the bone marrow [1–4, 123–125].

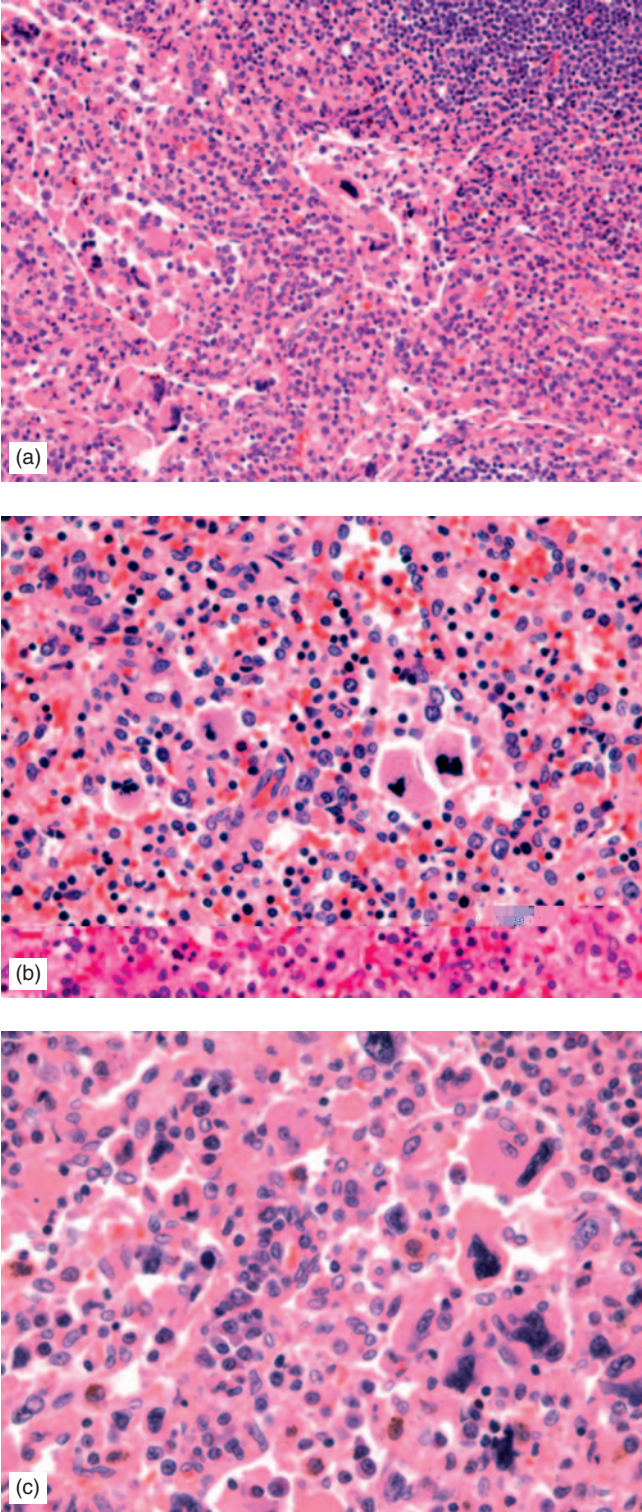


FIGURE 9.29 Lymph node: (a) low power, (b) high power, and spleen (c) sections demonstrating extramedullary hematopoiesis with numerous megakaryocytes and erythroid precursors.

Splenomegaly, thrombosis, and/or hemorrhagic events are frequent clinical manifestations. ET is diagnosed by the exclusion of reactive thrombocytosis (Table 9.6), familial thrombocytosis, and other subtypes of CMPD. The WHO criteria for the diagnosis of ET are presented in Table 9.7.

TABLE 9.6 Major differences between essential thrombocythosis (ET) and reactive thrombocytosis (RT).*

Features	ET	RT
Underlying disorder (inflammation, infection, malignancy, ischemia, tissue damage)	No	Yes
Digital or cerebrovascular ischemia	Yes	No
Arterial or venous thrombosis	Increased risk	No
Bleeding complications	Increased risk	No
Splenomegaly	May be present (40%)	No
Iron deficiency	No	May be present
Platelet function	May be abnormal	Normal
Large atypical megakaryocytes	Yes	No
Cytogenetic abnormalities	May be present	No
Plasma IL-6	Low	High
Plasma C-reactive protein	Low or normal	High
Spontaneous colony formation	Yes	No

*Adapted from Naeim, F. (1988). *Pathology of Bone Marrow*, 2nd ed. Williams & Wilkins, Baltimore and from Ref. [133].

TABLE 9.7 WHO criteria for the diagnosis of essential thrombocythemia.*

Positive criteria
1. Sustained platelet count of >600,000/ μ L in the peripheral blood.
2. Megakaryocytosis with the presence of enlarged forms in the bone marrow.
Criteria of exclusion
1. No evidence of polycythemia vera.
2. No evidence of chronic idiopathic myelofibrosis (lack of collagen fibrosis, minimal or lack of reticulin fibrosis).
3. No evidence of CML (no <i>Ph</i> ¹ or <i>BCR/ABL</i> fusion gene).
4. No evidence of myelodysplastic syndrome [no del(5q), t(3;3) or inv(3q); no dysgranulopoiesis; few or lack of micromegakaryocytes].
5. No evidence of reactive thrombocytosis (no underlying inflammation, infection, cancer or tissue damage; no iron deficiency and no history of splenectomy).

*Adapted from Ref. [1].

Etiology and Pathogenesis

The etiology and pathogenesis of ET are not known. A number of genes such as polycythemia rubra vera-1 (*PRV-1*),

TPO and its receptor *c-MPL* (myeloproliferative leukemia virus oncogene) have been implicated in the pathogenesis of ET, but so far have not been conclusive [126]. Unlike ET, mutations of *TPO* or *c-MPL* have been associated with the familial ET, which is an autosomal dominant disorder. The presence of *JAK2* mutation in ET patients has been correlated to a higher frequency of transformation to PV in some studies [127].

Several approaches based on the X-linked DNA and transcript analysis have demonstrated clonal involvement development of ET [128]. However, more recent studies suggest heterogeneity in this process with the involvement of stem cells at different levels and evidence of polyclonal hematopoiesis in up to 50% of the cases [124, 129].

Pathology

Morphology

The bone marrow morphologic findings are highly variable and often demonstrate overlapping features with other MPD [1–4]. The morphologic hallmarks are lack or minimal reticulin fibrosis and increased number of megakaryocytes, including large or giant forms (Figures 9.30–9.33). These megakaryocytes may show hyperlobulated nuclei and/or appear in clusters or diffusely dispersed. The megakaryocytic clusters may be found around the sinusoids or close to the bone trabeculae. The bone marrow is usually normocellular for age or moderately hypercellular, but occasionally hypocellular.

The bone marrow smears show increased number of large megakaryocytes and the presence of platelet aggregates. Emperipolesis (internalization of hematopoietic cells) is a frequent finding in megakaryocytes (Figure 9.31). There is no evidence of increased myeloblasts or significant dysplastic changes in the granulocytic or erythroid lineages. Some cases may show marked erythroid preponderance mimicking PV.

The blood smears show marked thrombocytosis with marked variation in platelet size and the presence of giant forms (Figure 9.32). Megakaryocytic fragments may be present. The white blood cell count is normal or slightly elevated with normal differential counts or mild granulocytosis. Rarely, early granulocytic forms may be present. Red blood cells are normochromic and normocytic. Dacrocytes (tear-drop-shaped red cells) and leukoerythroblastosis are not characteristic features of ET.

In summary, morphologic findings in ET are not specific and may be seen in reactive thrombocytosis as well as other myeloproliferative disorders, particularly PV and cellular phase of CIMP. In a large recent study by the Polycythemia Vera Study Group (PVSG), the conclusion was that histologic criteria described in the WHO classification are difficult to apply to distinguish ET from prefibrotic myelofibrosis [130].

Immunophenotypic Studies

No pathognomonic immunophenotypic features have been described in the blood or bone marrow of patients with ET.

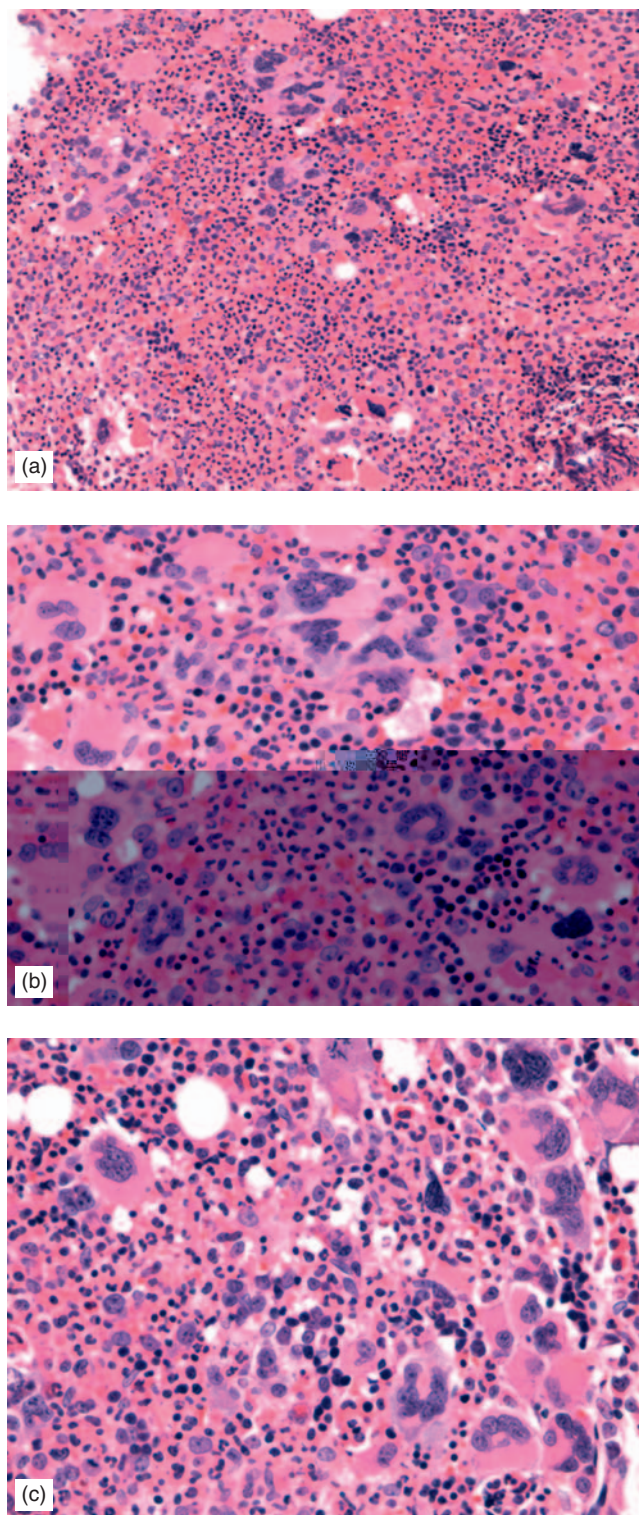


FIGURE 9.30 Bone marrow biopsy sections of a patient with ET demonstrating marked hypercellularity with clusters of megakaryocytes, including large and bizarre forms: (a) low power, (b), and (c) high power.

Molecular Studies

There are no specific molecular markers for ET, but the V617F mutation in *JAK2* has been found as in other MPD,

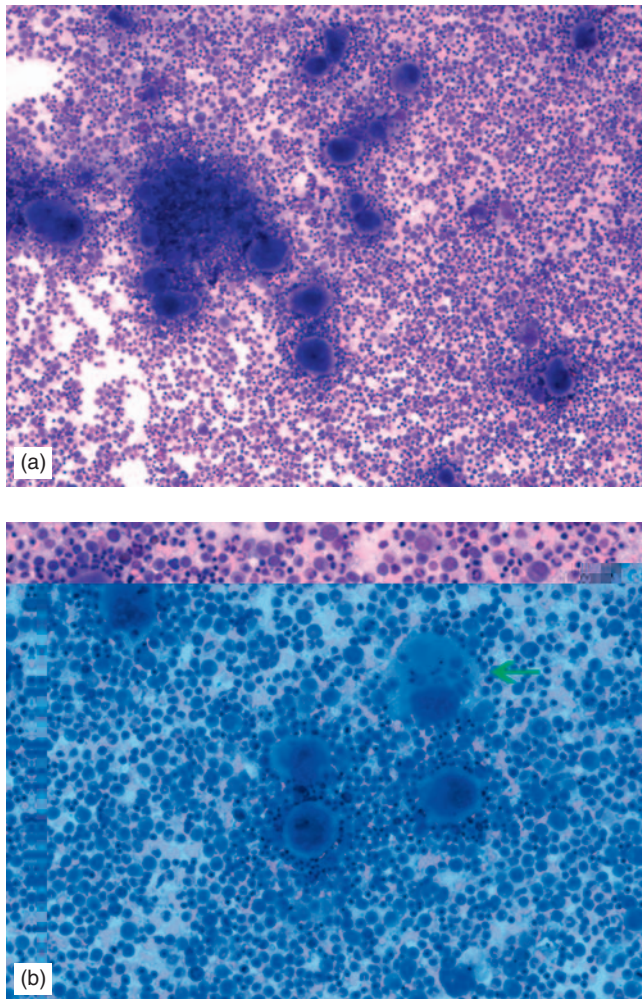


FIGURE 9.31 Bone marrow smears of a patient with ET demonstrating aggregates of megakaryocytes (a) low power and megakaryocytes with emperipolesis (b, arrow).

though not as frequently as in PV, typically ranging from 30% to 60% [130, 131]. In the rare familial cases, sequencing of the TPO or TPO-receptor genes to search for inherited mutations may be useful [132].

Cytogenetic Results

No consistent chromosomal anomaly was associated with ET, but chromosomal abnormalities have been observed in about 5–7% of patients. Anomalies in ET may have developed as a consequence of therapy or as a *de novo* leukemic clone [15, 17]. However, one group detected a low percentage (<10%) of trisomy 8 and/or 9 in about 55% of their patients by FISH [18].

Clinical Aspects

The incidence of ET is approximately 2.5 per 100,000 with a median age of about 60 years. Women are affected more than men with a female to male ratio of about 2. Up to 50% of the patients are asymptomatic. The remaining patients may show splenomegaly (25–40%) and/or other clinical symptoms

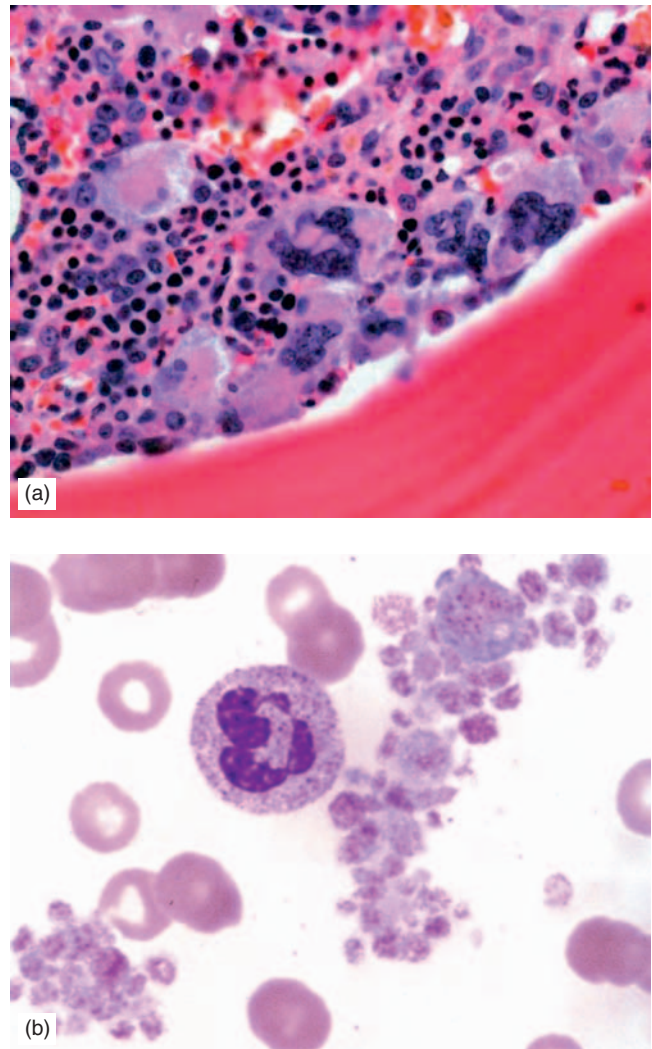


FIGURE 9.32 Bone marrow biopsy section of a patient with ET demonstrating a paratrabecular megakaryocytic aggregate (a) and platelet aggregates in blood smear (b).

such as headache, lightheadedness, syncope, erythromelalgia, transient visual disturbances, and thrombohemorrhagic incidents (15–25%). A history of prior thrombosis has an adverse prognostic value. Some ET patients with markedly elevated platelet counts may show acquired von Willebrand deficiency with an increased tendency of bleeding. ET in approximately 2–4% of the patients may transform to PV, CIMF, or AML after a median follow-up of about 10 years [124, 125, 133–135].

Hydroxyurea, anagrelide, interferon, and pipobroman are the agents most frequently used for the treatment of ET to reduce the platelet count and the risk of thrombosis [124, 125].

DIFFERENTIAL DIAGNOSIS

At the beginning of this chapter we displayed a long list of morphologic findings shared by various types of CMPD

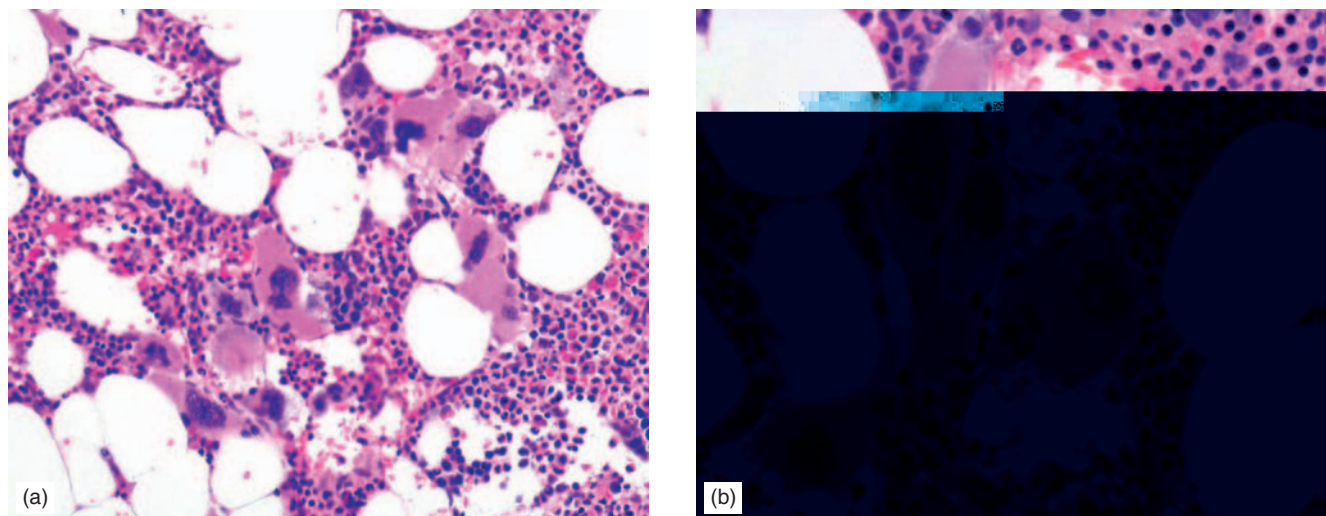


FIGURE 9.33 Bone marrow biopsy sections of a patient with ET demonstrating increased megakaryocytes, including large and bizarre forms: (a) low power and (b) high power.

TABLE 9.8 The major clinicopathologic features in chronic myeloproliferative disorders.*

Findings	PV	ET	CIMF	CML	CNL	CEL
<i>Bone marrow</i>						
Hypercellularity	+	±	Variable	+++	+	+
Increased myelopoiesis	+	—	+	+++	+++	+++
Increased erythropoiesis	+	—	±	—	—	—
Increased megakaryocytes	+	+	+	+	+	—
Fibrosis	Variable	Minimal	+ to + + +	+	—	—
Osteosclerosis	—	—	+	+	—	—
<i>Peripheral blood</i>						
Increased WBC	+	+	++	+++	+++	++
Basophilia	±	±	+	++	—	—
Eosinophilia	±	±	±	+	—	+++
RBC abnormalities	±	±	+++	±	—	—
Leukoerythroblastosis	—	—	+++	+	—	—
Thrombocytosis	+	+++	±	+	±	±
Abnormal platelets	±	+++	++	++	±	±
LAP (NAP) score	Elevated	Normal	Elevated	Low	Elevated	?
<i>Splenomegaly</i>	+	+	++ + +	+++	+	±
<i>Cytogenetics</i>	+8, +9, del(20q)	+8, +9	+8, +9, del(20q)	t(9;22)	+8, +9	t(5;12)
<i>Molecular studies</i>	JAK2	JAK2	JAK2	BCR-ABL	JAK2	PDGFRA

*Adapted from Ref. [3].

[1, 136, 137]. Diagnosis of several CMPD subtypes, such as CNL, CEL, and ET, are based on the exclusion of a wide variety of reactive conditions as well as other subtypes of CMPD (see Tables 9.4, 9.5, and 9.7). A summary of the distinctive features of CMPD subtypes is presented in Table 9.8). Following are pathologic features that may help to distinguish the four major different types of CMPD: PV, CML, CIMF, and ET.

Bone Marrow Findings

Fibrosis is the hallmark of the advanced stage of CIMF, while it is absent or minimal in ET. Other types of CMPD may show various degrees of fibrosis. Development of marrow fibrosis in CML may indicate an AP or blast transformation.

Megakaryocytes are commonly increased and show abnormal morphology in various subtypes of CMPD.

Dwarf forms or micromegakaryocytes are frequent in CML, giant megakaryocytes with frequent emperipolesis are common in ET, and dysplastic clusters of megakaryocytes are frequently found in the dilated sinusoids in early stages of CIMEF.

Eosinophilia and **basophilia** are most common in CML and least frequent in ET and PV. Progressive increase in basophils in CML patients is suggestive of an AP.

Peripheral Blood Findings

Leukoerythroblastosis is considered the morphologic hallmark of CIMEF, but it may also be seen in CML in chronic, accelerated, or blastic phases. Leukoerythroblastosis is not a feature of PV or ET.

Thrombocytosis is a frequent finding in all types of CMPD, but its persistent elevation $\geq 600,000/\mu\text{L}$ is more characteristic of ET. Giant platelets and megakaryocytic fragments have been observed more frequently in ET than in the others.

Granulocytosis is marked ($>50,000/\mu\text{L}$) and left-shifted with a significant number of myelocytes and metamyelocytes in CML. It is mild to moderate, but often left-shifted in CIMEF, and less frequently in PV or ET.

Dacrococytes (tear-drop-shaped red blood cells) are the morphologic indicators of bone marrow fibrosis and, therefore, the most frequent finding in CIMEF. Dacrocytosis is often associated with other red blood cell morphologic abnormalities, such as anisopoikilocytosis and the presence of schistocytes (fragmented red blood cells).

Molecular and Cytogenetics

At the molecular level, the finding of a *BCR-ABL1* fusion gene can help to separate CML from the other conditions. Conversely, detection of the *JAK2* mutation can distinguish PV, ET, and other non-CML MPDs from CML. Molecular methods that allow specific subtyping of the three *BCR-ABL1* isoforms (p190, p210, and p230) can assist in distinguishing ALL from the blast crisis of CML, as well as rare conditions such as CNL.

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Myelodysplastic/ Myeloproliferative Diseases

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The myelodysplastic/myeloproliferative disorders (MDS/MPD) are a group of hematologic disorders distinguished by clonal expansion of abnormal hematopoietic stem cells that share clinicopathological features of both MDS and chronic myeloproliferative disorders (CMPD) [1–4]. They are characterized by hypercellular bone marrows with excessive terminal proliferation of one or more hematopoietic lineages as well as dysplastic changes. This combination of proliferation and dysplasia may lead to increased production of one or more lineages (cytosis) and decreased production of other lineages simultaneously. Myeloid preponderance and left shift are common bone marrow findings, but blast cells are <20% in the bone marrow and/or the peripheral blood samples.

According to the WHO classification, these disorders are divided into the following major groups [1]:

- Chronic myelomonocytic leukemia
- Atypical chronic myeloid leukemia
- Juvenile myelomonocytic leukemia
- MDS/MPD, unclassifiable.

CHRONIC MYELOMONOCYTIC LEUKEMIA

Chronic myelomonocytic leukemia (CMML) is a clonal hematopoietic disorder characterized by both dysplastic and proliferative features including persistent monocytosis ($>1,000/\mu\text{L}$) of at least 3 months, bone marrow hypercellularity with myeloid preponderance, myelomonocytic

dysplasia and left shift with <20% blasts (including promonocytes) in the peripheral blood or bone marrow. Cytogenetic and molecular studies are negative for *Ph¹* and/or *BCR/ABL1* fusion gene (Table 10.1) [1–5].

Etiology and Pathogenesis

The etiology of CMML is not known. Most CMML cases lack cytogenetic or molecular changes that could explain the pathophysiology of this disorder. A small proportion of patients with CMML (about 2–3%), particularly

TABLE 10.1 The WHO proposed criteria for the diagnosis of chronic myelomonocytic leukemia.*

1. Peripheral blood monocytosis $>1,000/\mu\text{L}$.
2. Presence of myeloid blasts and promonocytes in the peripheral blood and/or the bone marrow; <20% of the differential counts.
3. No *Ph¹* or *BCR/ABL* fusion gene.
4. Dysplastic myelopoiesis. If myelodysplasia is minimal or absent:
 - (a) presence of an acquired clonal cytogenetic abnormality, or
 - (b) persistent monocytosis for at least 3 months, and
 - (c) exclusion of all other causes of monocytosis.

*From Ref. [1].

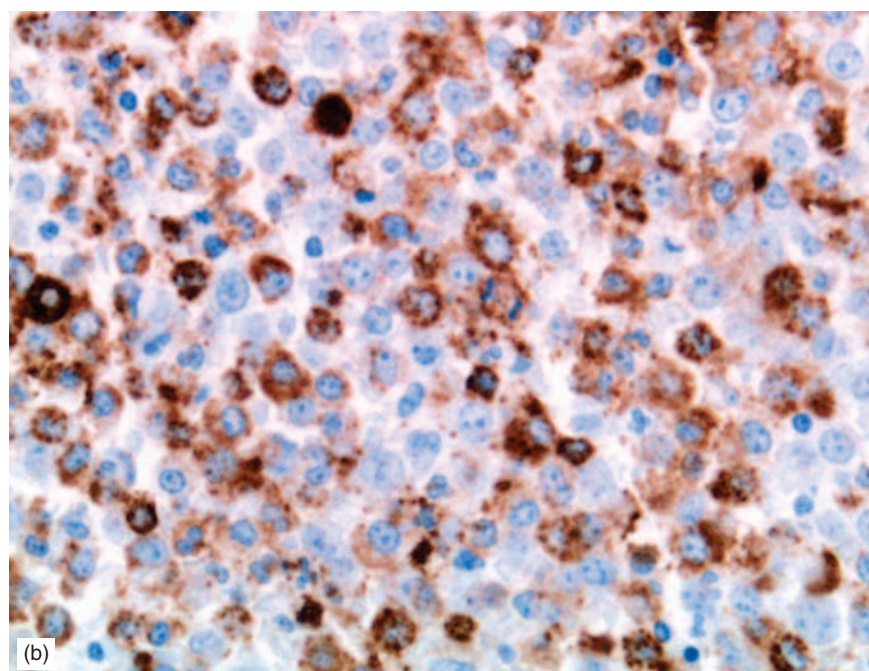
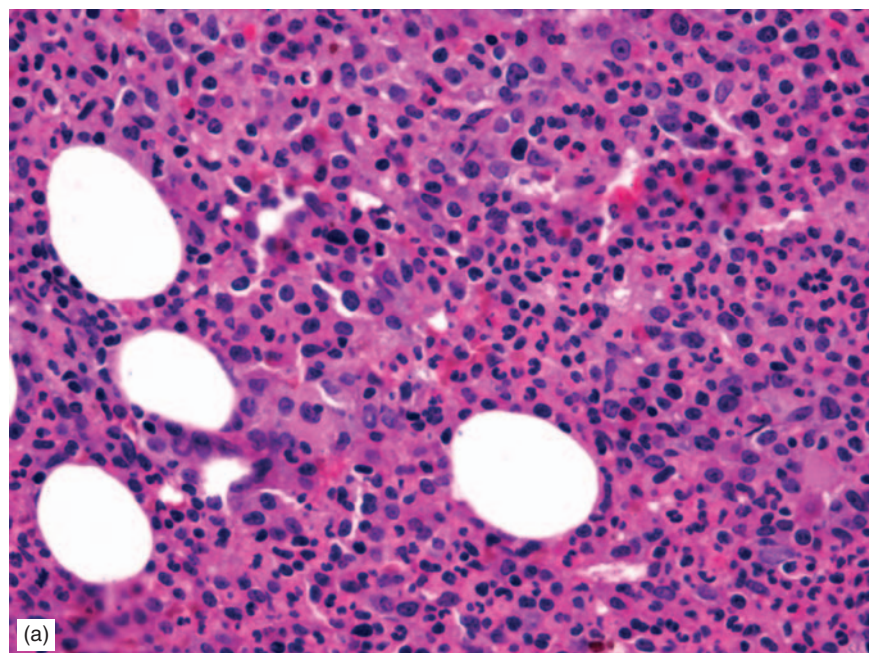


FIGURE 10.1 Bone marrow biopsy section from a patient with CMML demonstrating a hypercellular marrow with myeloid preponderance (a). The immunohistochemical stain for CD68 shows numerous positive monocytic/histiocytic cells (b).

in cases with eosinophilia, demonstrate $t(5;12)(q33;p13)$ [6]. This translocation leads to the fusion of the *TEL* and platelet-derived growth factor receptor (*PDGFβR*) genes. *K-RAS* and *N-RAS* mutations are reported in approximately 50% of CMML cases [7, 8]. Elevated levels of survivin (inhibitor of apoptosis) have been detected in patients with CMML [9]. A spontaneous *in vitro* GM colony formation has been frequently observed, and there is evidence of supra-normal granulocyte/macrophage colony forming units (CFU-GM) in CMML patients [5].

Pathology

Morphology

The bone marrow biopsy sections are mostly (>75%) hypercellular and display myeloid preponderance and left shift with increased number of immature myelomonocytic precursors (Figure 10.1) [1–3, 10–12]. There are reports of relatively frequent monocytic nodules in the bone marrow biopsy sections in patients with CMML (Figure 10.2) [13, 14]. Bone marrow fibrosis is reported in about one-third of the patients.

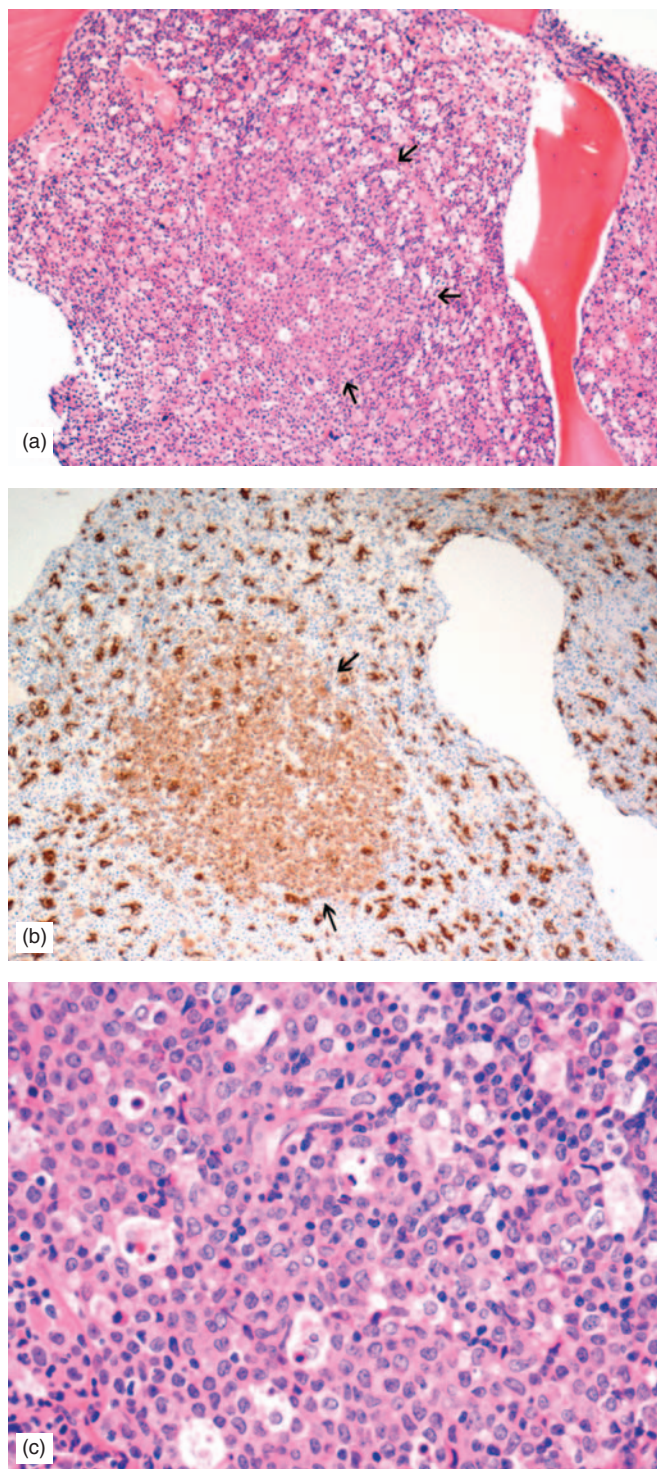


FIGURE 10.2 Monocytic nodules have been observed in bone marrow biopsy sections of patients with CMML. (a and b) represent low and high power fields (H&E stains). Arrow is pointed toward the monocytic nodule (b). The monocytic nodule is highlighted by immunohistochemical stain for CD68 (c, arrow). Courtesy of Sophie Song, M.D., Ph.D., Department of Pathology, UCLA.

The morphologic identification of monocytic precursors is much easier in bone marrow aspirate smears than in biopsy sections. The bone marrow smears often show dysplastic changes in the monocytic and the granulocytic series,

such as bizarre morphology, nuclear hyper- or hyposegmentation, and cytoplasmic hypo- or hypergranularity (Figure 10.3). The total number of myeloblasts, monoblasts, and promonocytes is <20%. Auer rods may be detected in some cases. Erythroid dysplasia such as megaloblastic changes, irregular nuclei, nuclear fragments, and ringed sideroblasts are frequently observed. Micromegakaryocytes are often present. Eosinophilia is observed in a small proportion of CMML cases, particularly in association with $t(5;12)(q33;p13)$ [1, 6].

The peripheral blood reveals monocytosis of $>1,000/\mu\text{L}$. In most instances the monocyte count is between 1,000 and 5,000/ μL , but occasionally it may exceed 50,000/ μL (Figure 10.3). CMML was divided into two myeloproliferative and myelodysplastic subtypes based on the WBC count [15]. There is often granulocytosis, with various degrees of anemia and/or thrombocytopenia. Monocytes and granulocytes are left-shifted and dysplastic with the presence of metamyelocytes, myelocytes, promyelocytes, and promonocytes (Figure 10.4a). The serum lysozyme levels are elevated. Myeloid blast cells and promonocytes are often <5%, but always <20% of the leukocyte differential count. Mild eosinophilia and/or basophilia are present. Usually, there is mild to moderate thrombocytopenia and anemia. Cases with over 13,000 leukocytes/ μL are considered of myeloproliferative subtype (Figure 10.5). However, significant clinical or biological differences between these two groups are debatable [1, 16–18].

Extramedullary involvement is observed in the spleen and sometimes in the liver and the lymph nodes. There seems to be a correlation between elevated WBC and splenomegaly. The myelomonocytic cells infiltrate into the splenic red pulp [19] and hepatic and lymph node sinuses.

The following subcategories are distinguished by the WHO [1]:

CMML-1: Blasts <5% in the peripheral blood and <10% in the bone marrow.

CMML-2: Blasts between 5% and 19% in the peripheral blood and/or between 10% and 19% in the bone marrow (Figure 10.6).

CMML with eosinophilia: When the eosinophil count is $>1,500/\mu\text{L}$. This category should be further divided into CMML-1 or CMML-2 according to the blast counts.

Immunophenotype and Cytochemical Stains

Flow cytometric studies of the peripheral blood or bone marrow often provide valuable information regarding (1) myelomonocytic precursors, (2) the presence of aberrant expression of CD molecules, and (3) the estimation of blast counts [20, 21].

Monocytes are positive for CD4, CD13, CD14, CD15, CD64, CD11 (b and c), HLA-DR, and may show aberrant expression of CD molecules, such as CD56 (Figure 10.7). Granulocytic cells are distinguished by coexpression of CD10, CD13, CD33, CD15, CD16, CD11c, and myeloperoxidase (MPO). They may lose some of these expressions, such as CD10 or CD33, and may show an abnormal dot plot clustering pattern due to hypogranularity. The blast population in the blood or bone marrow is detected as a dimly CD45-positive cluster which may express

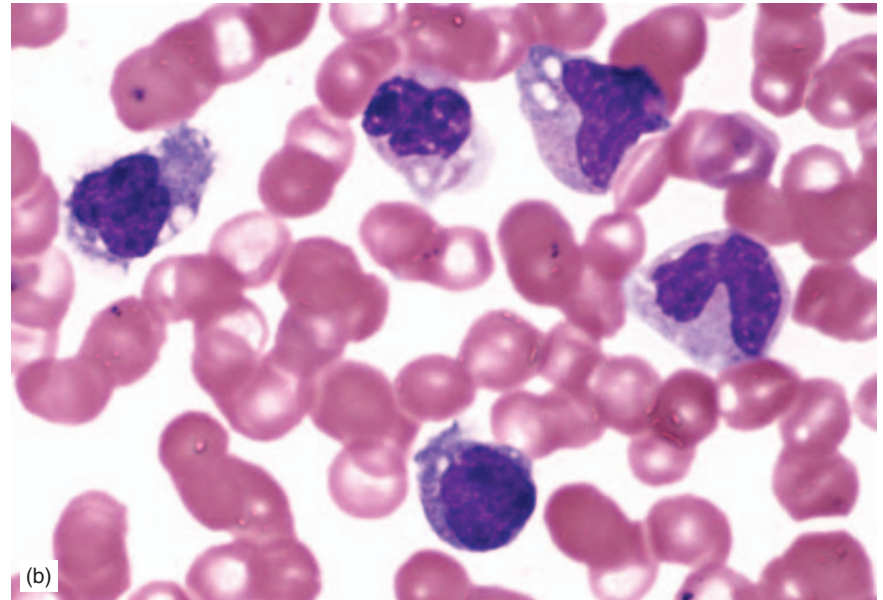
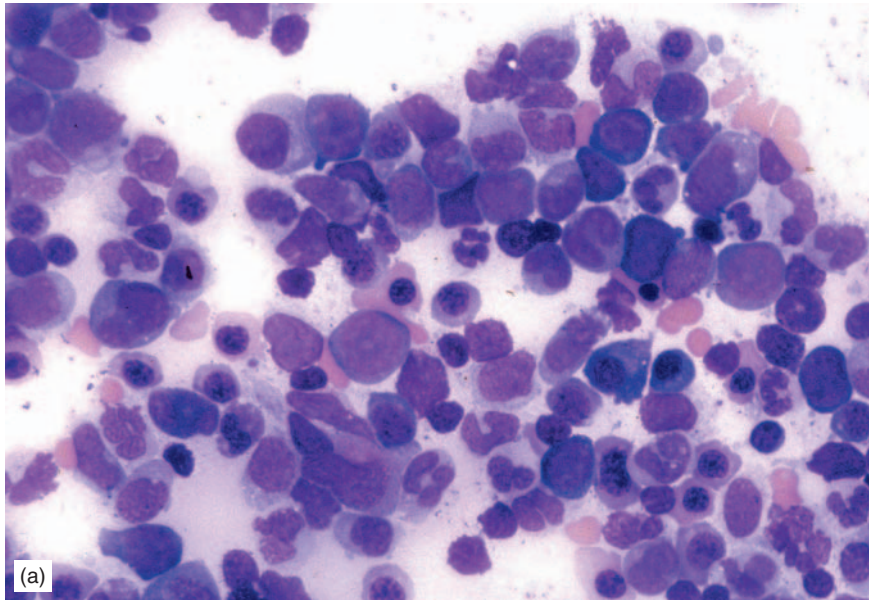


FIGURE 10.3 Bone marrow smears in patients with CMML demonstrate increased number of monocytes and promonocytes with dysplastic features (a). Blood smears reveal several monocytes with vacuolated cytoplasm and scattered, fine cytoplasmic azurophilic granules (b).

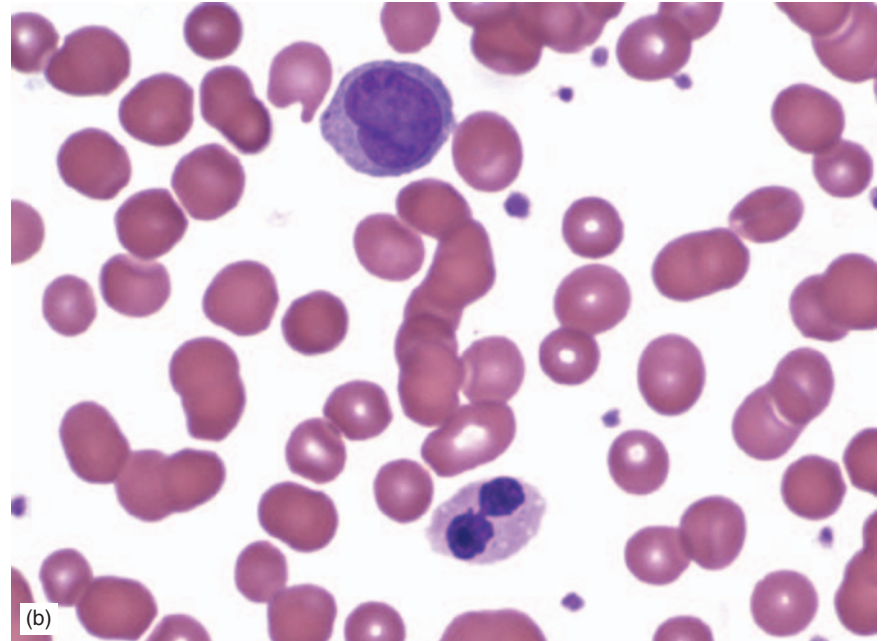
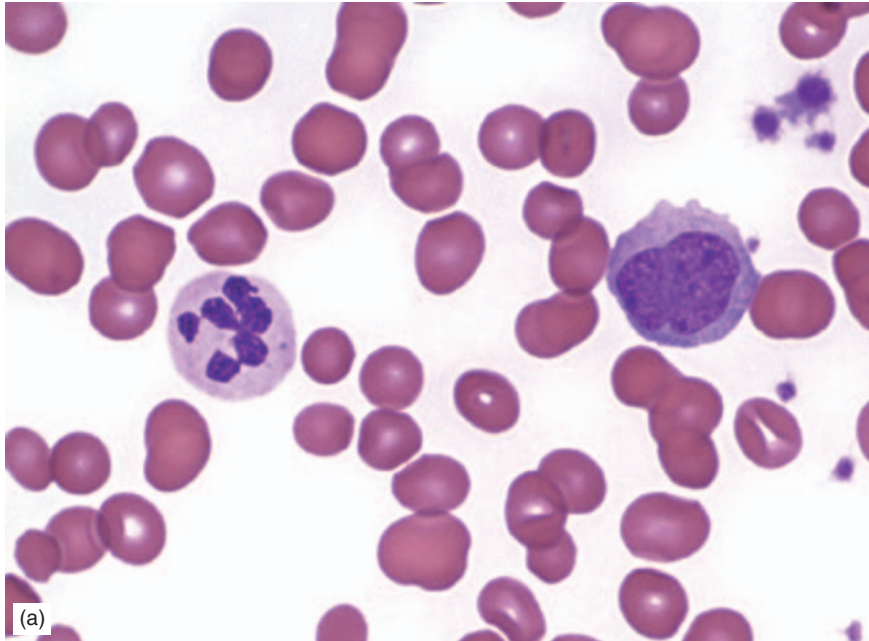


FIGURE 10.4 Dysplastic granulocytes are often present in the blood smears of patients with CMML. A hypersegmented and hypogranular neutrophil is demonstrated in (a) and a hyposegmented hypogranular neutrophil is depicted in (b).

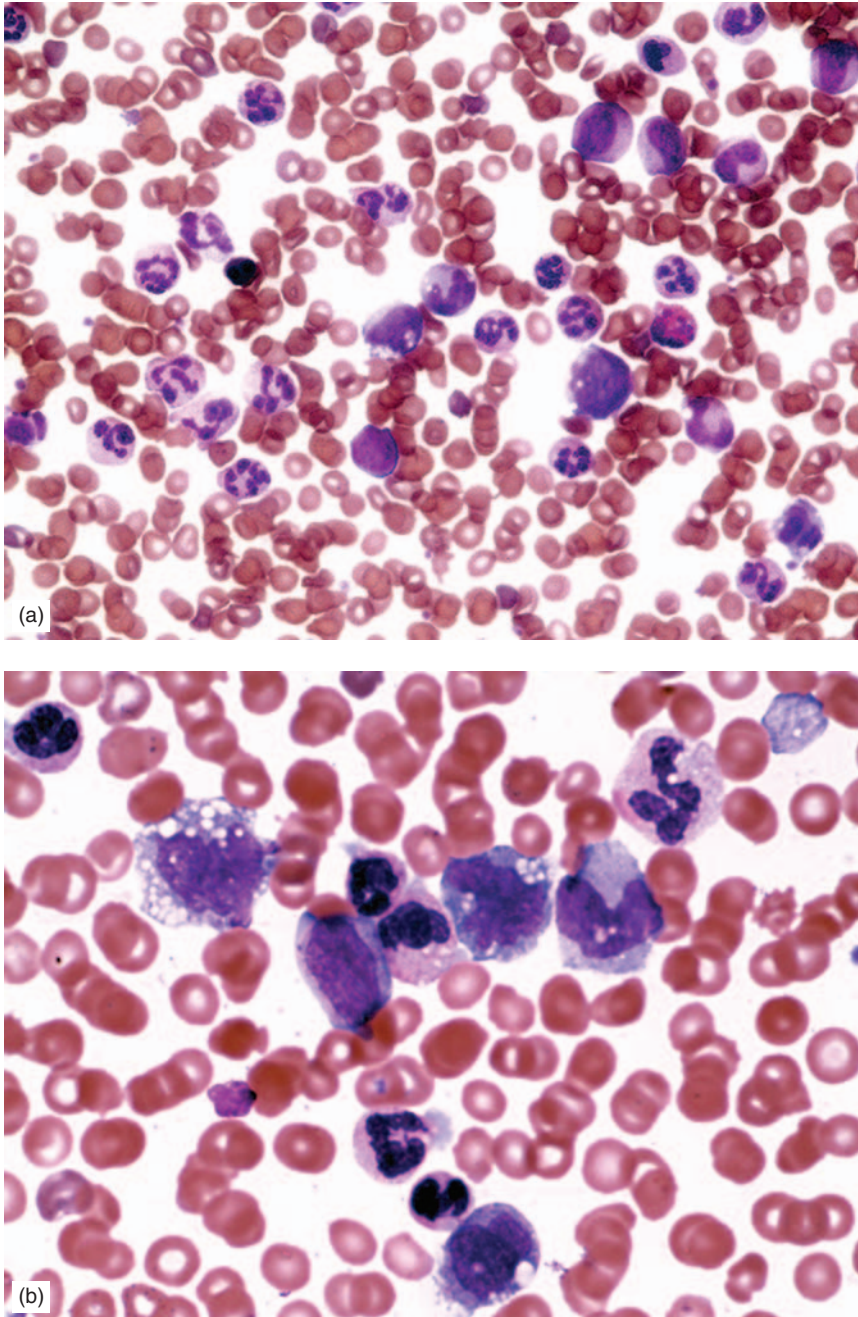


FIGURE 10.5 (a) Low and (b) high power field of a blood smear demonstrating the hyperproliferative variant of CMML.

CD34 and/or CD117 as well as other granulocytic- and/or monocytic-associated molecules.

Immunohistochemical stains, such as stains for glycoporphin A, hemoglobin A, MPO, CD68, lysozyme, CD34, and CD117, provide information regarding the M:E ratio, monocytic component, and increased blasts (Figure 10.8).

Cytochemical stains, such as MPO and non-specific esterase, are sometimes useful for estimation of the granulocytic and monocytic components and the lineage confirmation of the blast cells.

Molecular and Cytogenetic Studies

At the molecular level, *K-RAS* and *N-RAS* mutations are reported in approximately 50% of the CMML cases [7, 8].

There is also a report of the elevated levels of *survivin* in patients with CMML [9]. Fusion of the *TEL* and *PDGFβR* genes [t(5;12)(q33;p13)] has been demonstrated in the CMML subtype with eosinophilia [6]. The gene mutation profiles in various clonal myelogenous disorders are presented in Figure 10.9. Results suggest that the molecular pathology of CMML is closer to MDS than CMPD.

Approximately 20–40% of the patients with CMML show cytogenetic abnormalities including +8 (Figure 10.10), -7, del(7q), i(7q), and structural abnormalities of 12p [1, 22]. Pentasomy of chromosome 8, trisomy of chromosome 19, monosomy of chromosome 15, isochromosome 14q, and t(1;3)(p36;q21) have been reported in occasional cases [23–27]. As mentioned earlier, t(5;12)(q33;p13) is

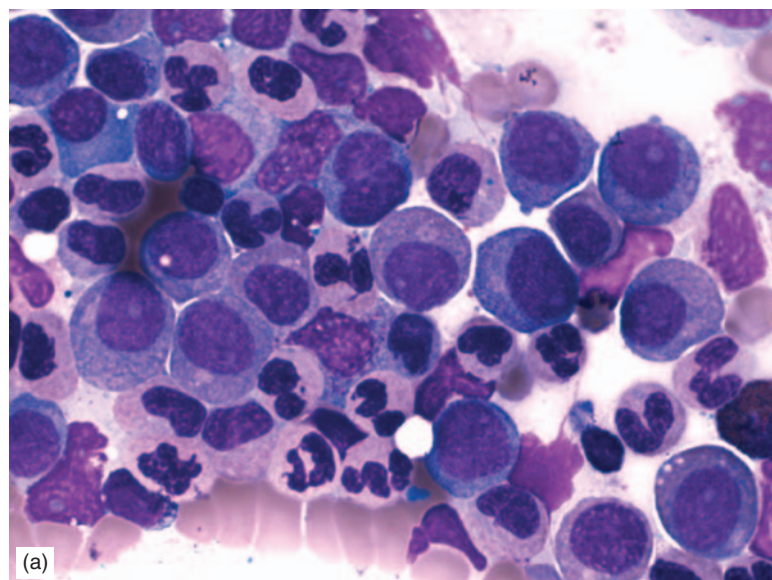
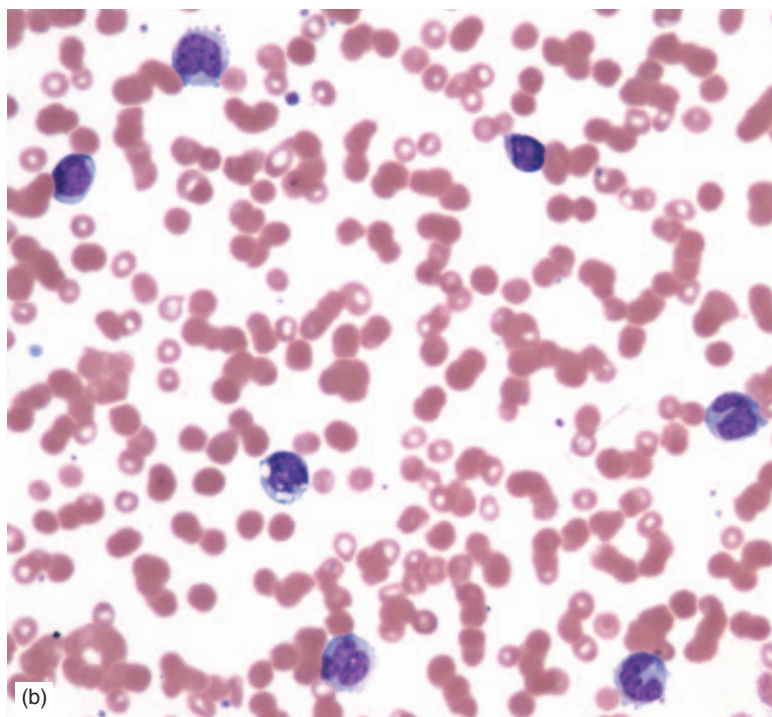


FIGURE 10.6 (a) Bone marrow and (b) blood smears representing CMML type 2.



frequently associated with CMML with eosinophilia [28, 29] (Figure 10.11). 11q23 abnormalities are infrequent.

Clinical Aspects

Chronic myelomonocytic leukemia is a disease of the elderly, with a median age of about 70 years. It affects men more than women, with a male:female ratio of 2–3:1 and an estimated incidence of about 4 per 100,000 per year [5, 30, 31]. Childhood CMML is relatively infrequent [32].

Clinical manifestations are related to anemia, thrombocytopenia, and splenomegaly. Some patients may demonstrate autoimmune disorders, such as vasculitis, pyoderma, and idiopathic thrombocytopenia [33]. Others may develop skin infiltration and serous effusions [34]. The reported unfavorable prognostic factors include low hemoglobin levels, low platelet counts, high percentage of marrow blasts ($>10\%$), lymphocytosis ($>2,500/\mu\text{L}$), elevated serum lactate dehydrogenase (LDH) and β_2 -microglobulin levels, and abnormal cytogenetics. Approximately 15–30% of CMML patients progress to acute leukemia [16, 35]. Evidence of

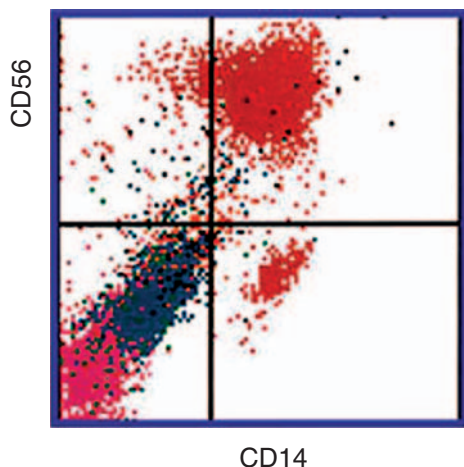


FIGURE 10.7 Flow cytometric analysis of monocytes in patients with CMML may show aberrant expression of CD56 or other markers. In this figure monocytes (red cluster, upper right quadrant) show coexpression of CD14 and CD56.

erythrophagocytosis has been suggested as an indicator of evolving blast transformation [36]. The median survival is about 2 years. Therapeutic approaches include conventional chemotherapy, such as hydroxycarbamide (hydroxyurea) and allogeneic bone marrow transplantation (BMT). Patients with $t(5;12)$ appear to respond to Gleevec. BMT is currently the only curative option with a 5-year survival rate of about 20%. The therapeutic trial of farnesyl transferase inhibitors (inactivating RAS protein) has opened an avenue in the area of targeted biological therapy [5].

ATYPICAL CHRONIC MYELOID LEUKEMIA

Atypical chronic myeloid leukemia (aCML) is a clonal hematopoietic disorder characterized by both dysplastic and proliferative features including persistent granulocytosis with left shift, bone marrow hypercellularity with dysplastic hematopoiesis, myeloid preponderance, and left shift [1, 37–39]. Cytogenetic and molecular studies are negative for Ph^1 and $BCR/ABL1$ fusion gene (Table 10.2). This disorder shows considerable overlapping of clinicopathological features with both CML and CMML. It is distinguished from CML by older median age, lower levels of granulocytosis, multilineage dysplasia, lack of basophilia, and absence of Ph^1 and $BCR/ABL1$ fusion gene. Unlike CMML, aCML shows no absolute monocytosis and a milder myeloid left shift.

Etiology and Pathogenesis

The etiology and pathogenesis of aCML are not known. Some investigators suggest that aCML and CMML are closely related and may represent different spectra of the same disorder [37, 38, 40]. Reports of $t(5;12)(q33;p13)$ and $t(5;10)(q33;q22)$ in occasional aCML cases raise the possibility of a pathogenic role for the $PDGF\beta R$ gene [37, 41].

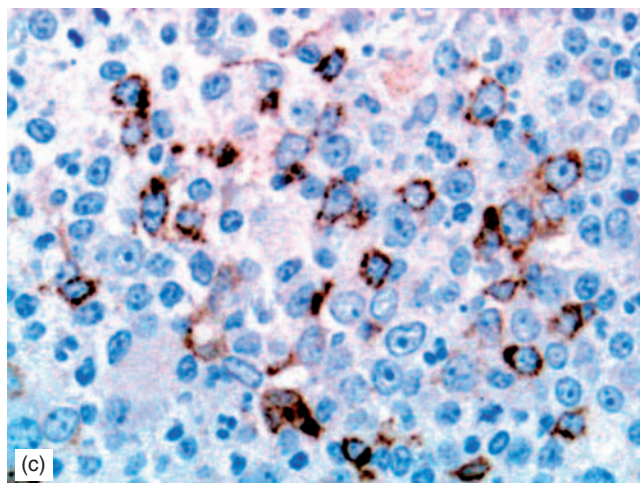
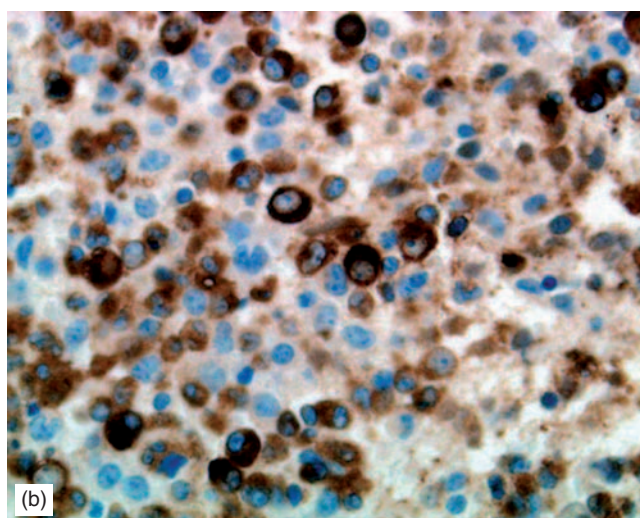
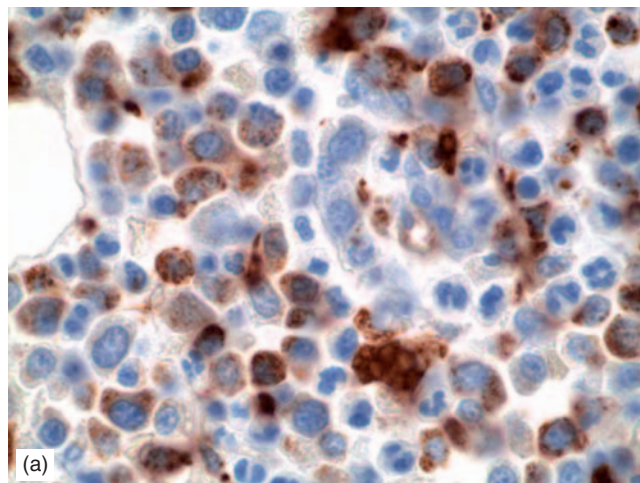


FIGURE 10.8 Immunohistochemical stains of a bone marrow biopsy involved with CMML demonstrating myelomonocytic precursors. Monocytes and macrophages express CD68 (a); myeloid precursors are strongly MPO-positive (b). Scattered CD34-positive cells represent blast cells (c).

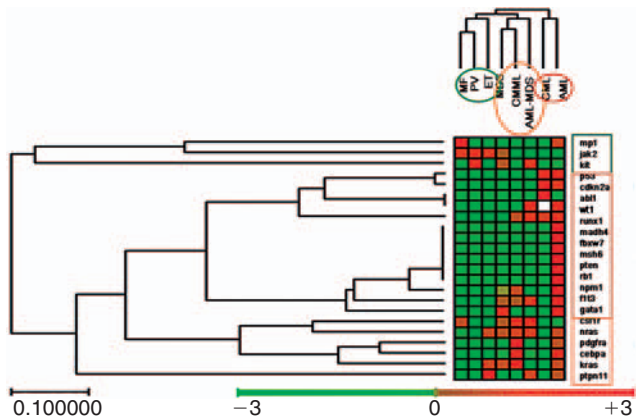


FIGURE 10.9 Schematic correlation of gene mutation profiles in various clonal myelogenous disorders. Data used from <http://www.sanger.ac.uk/genetics/CGP/cosmic>. MF: myelofibrosis, PV: polycythemia vera, ET: essential thrombocythemia, MDS: myelodysplastic syndrome, CMML: chronic myelomonocytic leukemia, AML: acute myeloid leukemia, and CML: chronic monocytic leukemia. Courtesy of Dejun Shen, M.D., Ph.D.

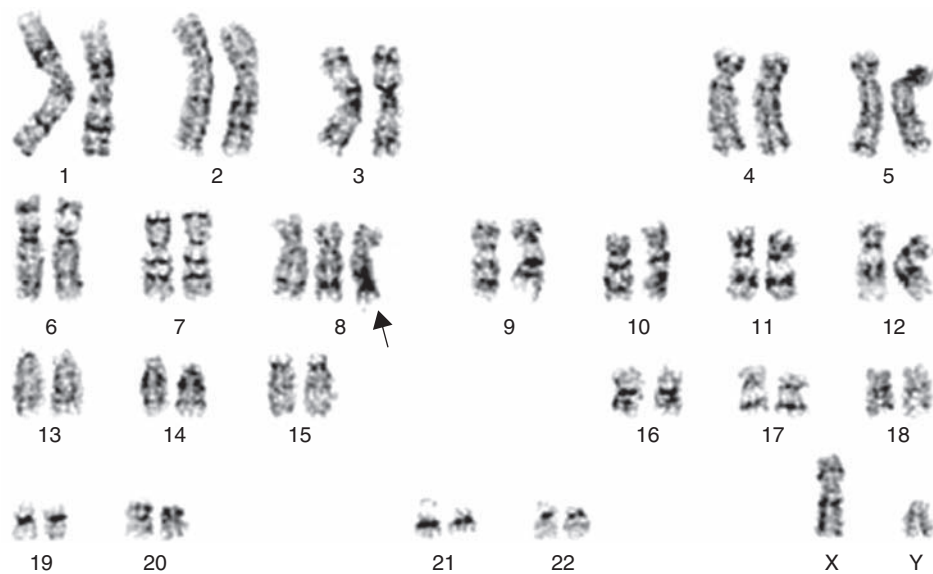


FIGURE 10.10 Trisomy 8 (arrow) in a patient with chronic myelomonocytic leukemia.

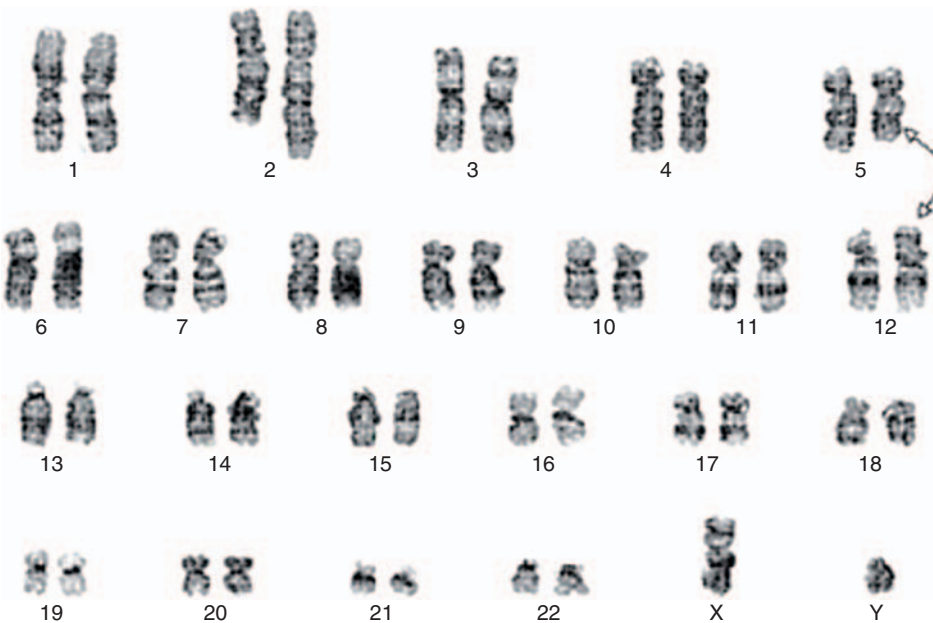


FIGURE 10.11 Translocation 5;12 (arrows) in a patient with chronic myelomonocytic leukemia.

TABLE 10.2 The WHO proposed criteria for the diagnosis of atypical chronic myeloid leukemia*

1.	Peripheral blood granulocytosis with increased number of mature and immature forms.
2.	Prominent dysgranulopoiesis.
3.	No <i>Ph</i> ¹ or <i>BCR/ABL</i> fusion gene.
4.	No or minimal absolute monocytosis.
5.	No or minimal absolute basophilia.
6.	Bone marrow hypercellularity with myeloid preponderance and left shift with dysgranulopoiesis, with or without other hematopoietic dysplasias.
7.	Fewer than 20% blasts in the blood or bone marrow.

*From Ref. [1].

Pathology

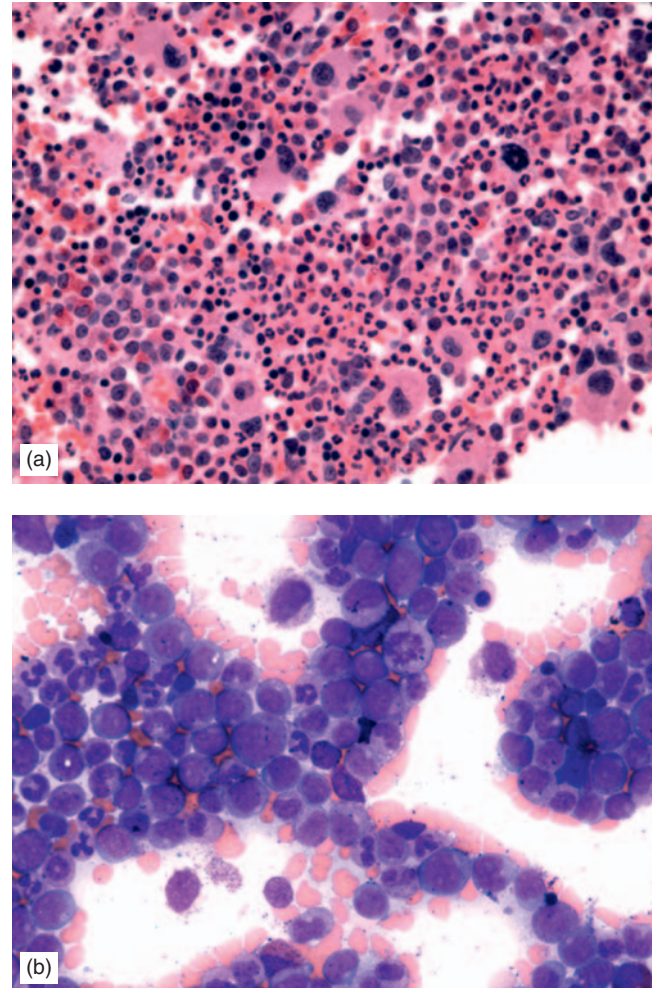
Morphology

The bone marrow biopsy and clot sections are hypercellular and show myeloid preponderance and left shift (Figure 10.12a). The bone marrow smears show an elevated M:E ratio, often >10:1 with dysgranulopoiesis and left shift. Hyposegmentation (pseudo Pelger-Huet) or hypersegmentation of the neutrophils, bizarre nuclear morphology of granulocytic precursors, and cytoplasmic hypo- or hypergranulation are frequently noted (Figure 10.12b) [1, 10–12]. Myeloblasts range from 1% to 10% but occasionally may reach up to 19%. Dyserythropoiesis with or without the presence of abnormal megakaryocytes are frequently observed. There is no evidence of monocytosis. The overall bone marrow morphologic features mimic those of CML, except for more significant dysplasia and lack of basophilia. Some cases may show increased reticulin fibers.

The peripheral blood shows elevated WBC, usually ranging from 30,000 to 90,000/ μ L, but in occasional cases exceeding 100,000/ μ L. The leukocytosis is primarily due to the increased number of neutrophilic granulocytes which are also left-shifted (Figure 10.12c). Granulocytic precursors account for about 10–20% or more of the leukocytes, but myeloblasts are always <10% and often range from 0% to 10%. Dysplastic granulopoiesis, as mentioned earlier, is always present. There is no or minimal absolute monocytosis and basophilia [1]. There is a variable degree of anemia which may be associated with abnormal morphology, such as anisopoikilocytosis and/or macrocytosis. Thrombocytopenia is a frequent feature.

Immunophenotype and Cytochemical Stains

No specific immunophenotypic features have been described for aCML. The leukocyte alkaline phosphatase (LAP) score is variable and ranges from low to high depending on the cases.

**FIGURE 10.12** Bone marrow biopsy section (a) and bone marrow smear (b) of patients with aCML show myeloid preponderance and left shift.

Molecular and Cytogenetic Studies

Atypical CML is negative for Philadelphia (*Ph*¹) chromosome and shows no evidence of *BCR-ABL1* rearrangement. A high frequency of *RAS* mutations is reported in bcr/abl-negative CML [42]. Occasional cases of aCML show t(5;12)(q33;p13) or t(5;10)(q33;q22) involving the *PDGF β R* gene [37, 41].

Clinical Aspects

Atypical CML is a disorder of older adults with apparently no sex predominance. The incidence of aCML is not yet established. Clinical manifestations, similar to CMML, are related to anemia, thrombocytopenia, and splenomegaly [1, 43, 44]. The median survival is <2 years with 20–40% chance of evolving to acute myeloid leukemia. Therapeutic approaches include conventional chemotherapy, such as hydroxycarbamide. Allogeneic BMT is potentially curative for eligible patients.

JUVENILE MYELOMONOCYTIC LEUKEMIA

Juvenile myelomonocytic leukemia (JMML) is a clonal hematopoietic disorder of early childhood characterized by hepatosplenomegaly, granulocytosis, and monocytosis with left shift and dysplastic changes, elevated hemoglobin F levels, and frequent skin involvement. JMML shares considerable pathologic features with CMML [1, 45–48]. It is relatively rare, usually affects children under the age of 4, and accounts for <2% of all hematologic malignancies in children [49].

Etiology and Pathogenesis

The etiology of JMML is not known. The deregulation of GM-CSF signal transduction through the *RAS* (retrovirus-associated sequence) pathway appears to play an important role in the pathogenesis of this disorder [50–52]. Mutations of *RAS* and the *NF1* genes have been reported in about 30% of patients with JMML [50–54]. The *NF1* gene encodes neurofibromin, a guanosine triphosphatase protein, which is a *RAS* inhibitor. Mutations in *PTPN11*, which encodes the protein tyrosine phosphatase Shp-2, are common in JMML. It has been suggested that these mutations may induce hypersensitivity of hematopoietic progenitors to GM-CSF [55].

Pathology

Morphology and Laboratory Findings

The WHO criteria for the diagnosis of JMML are presented in Table 10.3 [1]. The bone marrow samples are cellular and display myeloid preponderance and left shift with increased number of immature myelomonocytic precursors (Figure 10.13) [1, 10–12]. However, the total number of myeloblasts, monoblasts, and promonocytes is <20%. Dysplastic changes

TABLE 10.3 The WHO proposed criteria for the diagnosis of juvenile myelomonocytic leukemia*.

1.	Peripheral blood monocytosis >1,000/ μ L.
2.	Presence of myeloid blasts and promonocytes in the peripheral blood and/or the bone marrow; <20% of the differential counts.
3.	No <i>Ph</i> ¹ or <i>BCR/ABL</i> fusion gene.
4.	Plus two or more of the following: (a) Elevated levels of hemoglobin F. (b) Presence of immature granulocytes in the peripheral blood. (c) WBC >10,000/ μ L. (d) Clonal chromosomal aberrations. (e) Hypersensitivity of myeloid precursors to GM-CSF <i>in vitro</i> .

*From Ref. [1].

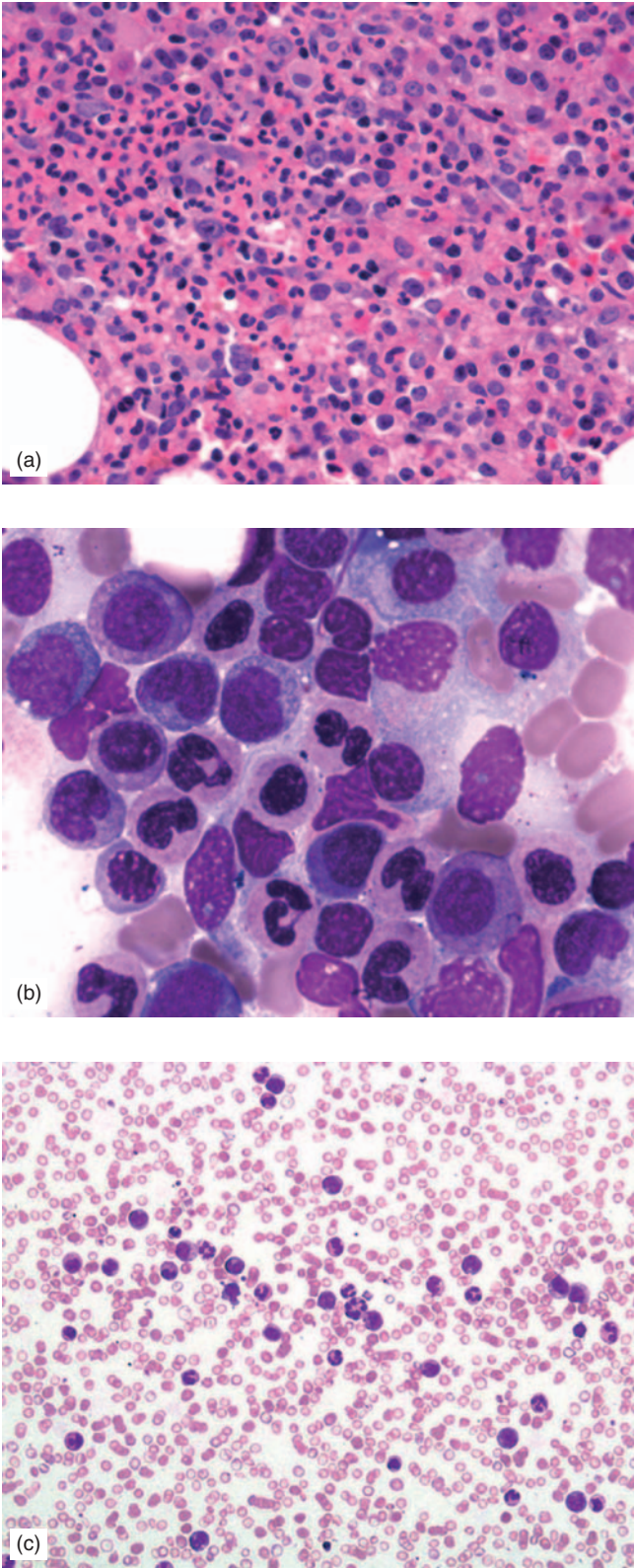


FIGURE 10.13 Morphologic features of JMML are very similar to CMML, characterized by the preponderance of myelomonocytic cells in the bone marrow (a: biopsy section; b: bone marrow smear) and absolute monocytosis in the peripheral blood (c).

are frequently observed in the monocytic and the granulocytic series, such as bizarre morphology, nuclear hyper- or hyposegmentation, and cytoplasmic hypo- or hypergranularity, but Auer rods are not present. Erythroid dysplasia is often minimal or lacking. Megakaryocytes may be reduced or show some degree of dysplastic changes including presence of micromegakaryocytes. Eosinophilia and basophilia are rare.

The peripheral blood reveals monocytosis and granulocytosis, often with various degrees of anemia and/or thrombocytopenia. Monocytes and granulocytes are left-shifted and dysplastic with the presence of metamyelocytes,

myelocytes, promyelocytes, and promonocytes (Figure 10.12c) [1, 10–12]. The serum lysozyme levels are elevated. Myeloid blast cells and promonocytes are often <5% and never >19% of the leukocyte differential count. The average leukocyte count is about 30,000/ μ L. There is often some degree of anisopoikilocytosis. Macrocytosis is a frequent feature and nucleated red blood cells are often present. Eosinophilia and basophilia are rare. The hemoglobin F levels are elevated, the glucose-6-phosphatase activity is increased, and there is a high incidence of antinuclear (50%) and anti-IgG (40%) antibodies. There may be evidence of polyclonal hypergammaglobulinemia [1].

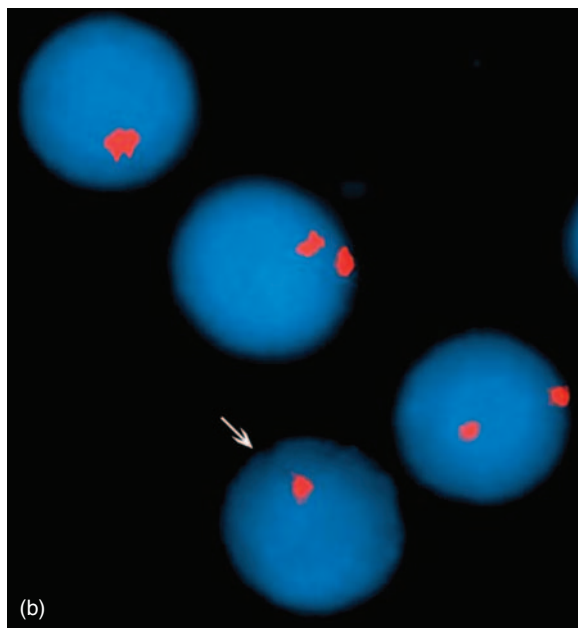
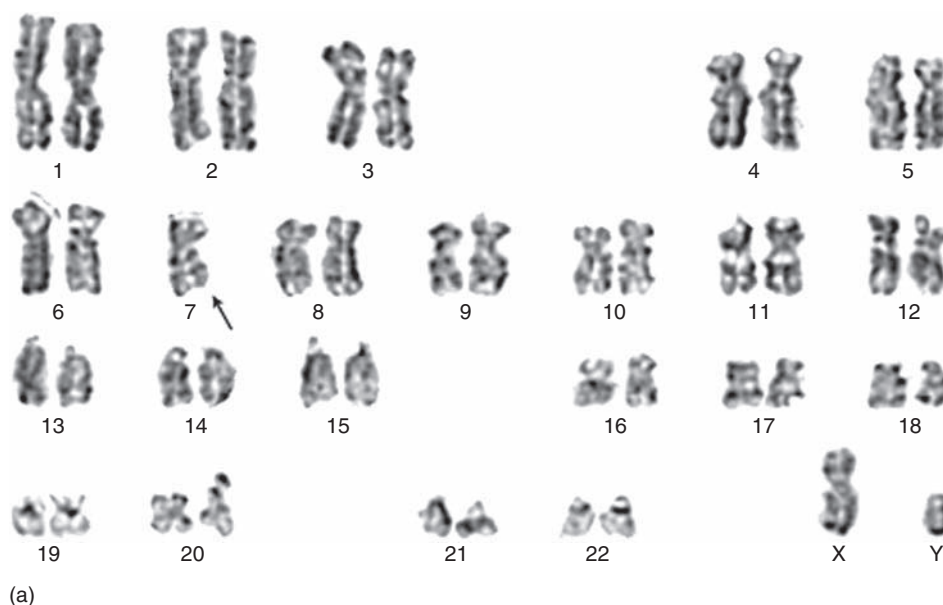


FIGURE 10.14 Monosomy 7 (arrow) in a child with JMML: (a) karyotype and (b) FISH.

TABLE 10.4 Clinicopathologic features of chronic myeloid leukemia (CML), atypical chronic myeloid leukemia (aCML), chronic myelomonocytic leukemia (CMML), and juvenile myelomonocytic leukemia (JMML).

Features	CML	aCML	CMML	JMML
Average age (year)	46	57	72	<4
Male:female	>1	>1	>1	>1
Splenomegaly	+++	++	+	+
<i>Blood</i>				
Average leukocyte count	>100,000/ μ L	60,000/ μ L	35,000/ μ L	30,000/ μ L
Absolute monocytosis	Often no	Often no	Yes	Yes
Basophilia	Often yes	No	No	No
Myeloid precursors	+++	++	++	++
LAP	Reduced	Variable	Variable	Variable
Anemia	Present	Present	Present	Present
Elevated hemoglobin F	No	No	No	Yes
Platelet count	Variable	Reduced	Reduced	Reduced
<i>Bone Marrow</i>				
Cellularity	Increased	Increased	Increased	Increased
Myeloid preponderance	Yes	Yes	Yes	Yes
Myeloid left shift	Yes	Yes	Yes	Yes
Monocytosis	No	No	Yes	Yes
Significant dysplasia	No	Yes	Yes	Yes

*Adapted from Refs [1, 37].

Extramedullary involvement is a frequent feature with the infiltration of the myelomonocytic cells in the dermis, the lung parenchyma, the hepatic sinusoids, and the splenic red pulp [1].

Immunophenotype and Cytochemical Stains

Similar to MDS and CMML, dysplastic myelomonocytic cells in JMML may show abnormal expression of CD molecules, such as aberrant expression of CD56 by monocytes or reduced expression of CD10 by neutrophils. The elevated number of monocytic cells in bone marrow and peripheral blood can be established by flow cytometry, immunohistochemistry, and/or cytochemical stains. Monocytes express CD4, CD14, CD16, HLA-DR, CD64, and CD68, and show strong positive reactions with lysozyme and non-specific esterase stains. LAP scores may be reduced.

Molecular and Cytogenetic Studies

As mentioned earlier, mutations of *RAS*, *NF1*, and *PTNP11* genes are frequently detected in patients with JMML [50–54, 56]. Recently, quantitative measurements of *RAS* and *PTNP11* were made by an allele-specific polymerase chain reaction (PCR) assay called TaqMan, and increased levels were correlated with relapse of JMML in transplanted patients [54]. Methylation of *p15*, which is a frequent finding in patients with MDS (78%), is a rare event (17%) in JMML patients [57].

Cytogenetic aberrations are non-specific. Monosomy of chromosome 7 is the most frequent cytogenetic abnormality (Figure 10.14) [58, 59]. Rare cases with t(3;12) (q21-22; p13.3) or der(15)t(3;15)(q13.1; q26) have been reported [60].

Clinical Aspects

Juvenile myelomonocytic leukemia is a rare early childhood hematologic disorder with roughly 0.6 new cases per year per million children at risk, accounting for <2% of hematologic malignancies in children [45–49]. The majority of patients are under the age of 4 years with a male:female ratio of about 2:5 [61–63]. Splenomegaly, hepatomegaly, lymphadenopathy, and skin rashes are noted in >90%, 80%, 70%, and 35% of the patients, respectively [61]. The cutaneous manifestations include neoplastic infiltration, eczema, xanthoma, and café-au-lait spots. JMML shows a high frequency (7–14%) of association with neurofibromatosis type 1 [45–49]. The prognosis is poor, but affected infants younger than 1 year of age appear to do better than older children [64]. Elevated hemoglobin F levels (>15%) and low platelet counts (<33,000/ μ L) are amongst the unfavorable prognostic indicators [64, 65].

Allogeneic BMT is the only available cure with approximately 50% 5-year event-free survival rate [66, 67].

Recent reports on the effects of zoledronic acid (ZOL), a blocker of RAS activity, are promising [52].

The entity *infantile (childhood) monosomy 7 syndrome* shares most of the clinicopathologic features of CMML [68–70]. Both disorders affect children at early ages (often <1 year old), show male predominance, show an association with neurofibromatosis type 1, and similar frequency of *RAS* gene mutation. Also, monosomy 7 is the most frequent chromosomal abnormality in JMML. However, children with JMML who lack monosomy 7 often display elevated levels of hemoglobin F. It seems that infantile monosomy 7 represents a cytogenetic subtype of JMML.

DIFFERENTIAL DIAGNOSIS

The chronic myeloproliferative/myelodysplastic disorders show significant overlapping of morphologic features among themselves and with CML. CML patients are usually younger and show much more severe leukocytosis than patients with CMML or aCML. Basophilia is a common feature in CML but not present in CMML or aCML [43]. Dysplastic myelopoiesis is a characteristic feature of aCML, CMML, and JMML, whereas it is insignificant in CML. Monocytosis is the hallmark of CMML and JMML and is lacking in CML and aCML. *Ph¹* and/or *BCR/ABL* fusion gene are present in CML but negative in aCML, CMML, and JMML. JMML is a disease of early childhood (usually under the age of 4) and is commonly associated with skin rashes and elevated hemoglobin F levels [49, 70, 71]. There is a high frequency (7–14%) of neurofibromatosis type 1 in JMML patients. The major clinicopathologic features of CMML, aCML, and JMML are compared with one another and with CML in Table 10.4.

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Acute Myeloid Leukemia

GENERAL CONSIDERATIONS

Acute myeloid leukemia (AML) represents a group of hematopoietic neoplasms derived from the bone marrow precursors of myeloid lineage. The neoplastic process is the result of clonal proliferation of an aberrant, committed stem cell at the level of CFU-S or later stages of differentiation leading to the accumulation of immature forms without, or with limited, maturation. Other terms used to denote AML include acute non-lymphoid leukemia (ANLL), acute myelogenous leukemia, and acute myeloblastic leukemia. The current WHO classification of AML is presented in Table 11.1 [1]. According to this classification, AML is divided into four major categories as:

1. AML with recurrent genetic abnormalities
2. AML with multilineage dysplasia
3. AML and myelodysplastic syndromes (MDS), therapy related
4. AML not otherwise categorized.

Etiology and Pathogenesis

The etiology of AML is not clearly understood. It has been demonstrated that environmental factors and family background play important roles in the development of AML. Three major environmental insults have been implicated in the increased incidence of AML: (1) ionizing radiation, (2) chemotherapeutic agents, and (3) occupational exposure to chemicals [1–6].

Ionizing radiation induces DNA damage leading to chromosomal breaks which may cause mutations, deletions, and translocations.

The extent of this damage depends on the type of radiation, the amount and rate of absorption, distribution of the absorbed energy in the tissue, and the intervals between the radiation exposures [7–10]. The incidence of AML in atomic bomb survivors has been estimated to be as high as 24-fold than in the control population. This increase in leukemia incidence in the atomic bomb survivors started to show up 3 years after the radiation exposure, reached its peak at 6–8 years, and leveled off after 20 years [11–13]. The cumulative mortality studies of patients irradiated for the treatment of ankylosing spondylitis have shown a 10-fold increase in the incidence of acute leukemia. Also, radiation therapy in malignancies such as Hodgkin lymphoma and thyroid cancer has been associated with a higher incidence of AML, particularly when radiation is administered in combination with chemotherapy [7, 8].

Alkylating agents and topoisomerase type II inhibitors are amongst the most potent chemical factors in the development of acute leukemia and make up the bulk of the subcategory of therapy-related AMLs (t-AMLs) in the WHO classification (discussed later). The cumulative risk of drug-induced AML has been reported to range from 10% to 17% within 4–9 years from the beginning of chemotherapy or the combination of chemotherapy and radiation in patients with plasma cell myeloma, ovarian cancer, or Hodgkin lymphoma [14–22]. The latency period of alkylating-induced AML is usually 4–6 years. The topoisomerase type II inhibitor-associated AML has an overall shorter latency period, usually <3 years (discussed later). Also, immunosuppressive therapy in transplant patients and in patients with immune-associated disorders may increase the risk of AML [23, 24].

Occupational exposure to petroleum products (such as benzene), insecticides, and other

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TABLE 11.1 Classification of AML according to the WHO.*

1.	AML with recurrent genetic abnormalities
(a)	AML with t(8;21)
(b)	AML with abnormal eosinophils and inv(16) or t(16;16)
(c)	Acute promyelocytic leukemia with t(15;17) or variants
(d)	AML with 11q23 (MLL) abnormalities
2.	AML with multilineage dysplasia
(a)	Following myelodysplastic syndrome or myelodysplastic/ myeloproliferative disorder
(b)	Without antecedent myelodysplastic syndrome
3.	AML and myelodysplastic syndrome, therapy related
(a)	Alkylating agent related
(b)	Topoisomerase type II inhibitor related
(c)	Other types
4.	AML not otherwise categorized
(a)	AML minimally differentiated
(b)	AML without maturation
(c)	AML with maturation
(d)	Acute myelomonocytic leukemia
(e)	Acute monoblastic and monocytic leukemia
(f)	Acute erythroid leukemia
(g)	Acute megakaryoblastic leukemia
(h)	Acute basophilic leukemia
(i)	Acute panmyelosis
(j)	Myeloid sarcoma

*Adapted from Ref. [1].

organic solvents increases the risk of AML [25–28]. The incidence of clonal chromosomal abnormalities is significantly higher in patients exposed to chemical solvents than the unexposed population. The most frequent aberrations include –5/del(5q), –7/del(7q), +8, and +21. Cigarette smoking, particularly in individuals over the age of 60, has shown a two-fold increase in the risk of AML [29–31].

Certain familial disorders are associated with a higher risk of AML. There is a 10- to 20-fold increased chance of leukemia, particularly AML, in patients with Down syndrome (trisomy 21) [32]. Many of these patients show an acquired mutation of the GATA-1 transcription factor which plays a role in megakaryocytic development. A significant proportion of AMLs in Down syndrome patients is of megakaryoblastic subtype. The incidence of AML is also high in inherited disorders with defective DNA repair, such as Bloom’s syndrome, Fanconi’s anemia, Wiscott–Aldrich syndrome, neurofibromatosis, Kostmann’s syndrome (infantile agranulocytosis), and Diamond–Blackfan anemia [33, 34]. A rare constitutional trisomy 8 syndrome (with dysmorphic facial features and abnormal skeletal muscles) has also been associated with AML [35, 36].

Although retroviruses have been demonstrated to play a role in leukemogenesis of AML in experimental animals, no clear association has been found between AML and retroviruses in humans. The two major mechanisms of leukemogenesis by retroviruses in animal models are [28, 37–42]:

1. Encoding an oncogene that leads to leukemic transformation

2. Inappropriate activation of expression of a gene adjacent to its integration site.

Leukemogenesis, similar to most other cancer developments, appears to be a multistep process involving structural and functional changes in a cascade of genes leading to the clonal expansion of defective stem cells. These genetic alterations often include mutations of oncogenes and/or loss of tumor suppressor genes. The specific genetic events in the process of leukemogenesis are not currently well understood, though it has been suggested that at least two mutations are required: one leading to a proliferative advantage and the other causing impairment of the maturation process (the “two-hit” hypothesis). The following examples represent the multistep concept of leukemogenesis in AML.

In the chronic phase of chronic myeloid leukemia (CML), leukemic cells show t(9;22) resulting in the *BCR/ABL1* fusion gene. As CML progresses to the accelerated phase and then blast transformation, additional genetic abnormalities, such as mutation of *p53* (a tumor suppressor gene), evolve.

The high frequency of AML in patients with MDS strongly supports the two-hit hypothesis for leukemogenesis. MDS represents the first step, or the first hit, with frequent detectable chromosomal aberrations, including –5/del(5q), –7/del(7q), and +8. Evolution to AML, often with additional molecular and/or cytogenetic changes, depicts the final stage, or the second hit. There are studies indicating that the remission bone marrow samples from AML patients with t(8;21)(q22;q22) and the *RUNX1/RUNXT1* fusion transcript carry the abnormal fusion for several years after the completion of chemotherapy. These observations suggest that the *RUNX1/RUNXT1* may not be sufficient for the development of AML by itself, and additional mutation(s) are necessary [37, 40, 43]. Some of the patients with severe congenital neutropenia and a documented nonsense mutation in the G-CSF receptor develop AML, raising the possibility that this kind of mutation causes resistance to apoptosis, allowing more time for additional mutation(s) to occur.

A significant proportion of AMLs are associated with specific recurrent cytogenetic abnormalities such as t(8;21)(q22;q22), inv(16)(p13q22), t(15;17)(q11;q12), and 11q23 abnormalities. The products of the fusion genes resulting from chromosomal rearrangements play an important role in the evolution of AML (discussed later).

Pathology

Morphology

Acute myeloid leukemia refers to neoplasm of non-lymphoid hematopoietic progenitor cells. Therefore, it consists of subtypes representing various myeloid differentiations such as myeloblasts, promyelocytes, monoblasts, promonocytes, erythroblasts, and megakaryoblasts [1–4, 44]. In general, myeloblasts are the most predominant precursor cells in AML categories. The WHO requirement for the diagnosis of AML is the presence of 20% or more blast cells in the bone marrow or blood differential counts [1]. In addition to myeloblasts, “blast” counts in certain categories of AML may include monoblasts, megakaryoblasts, promonocytes,

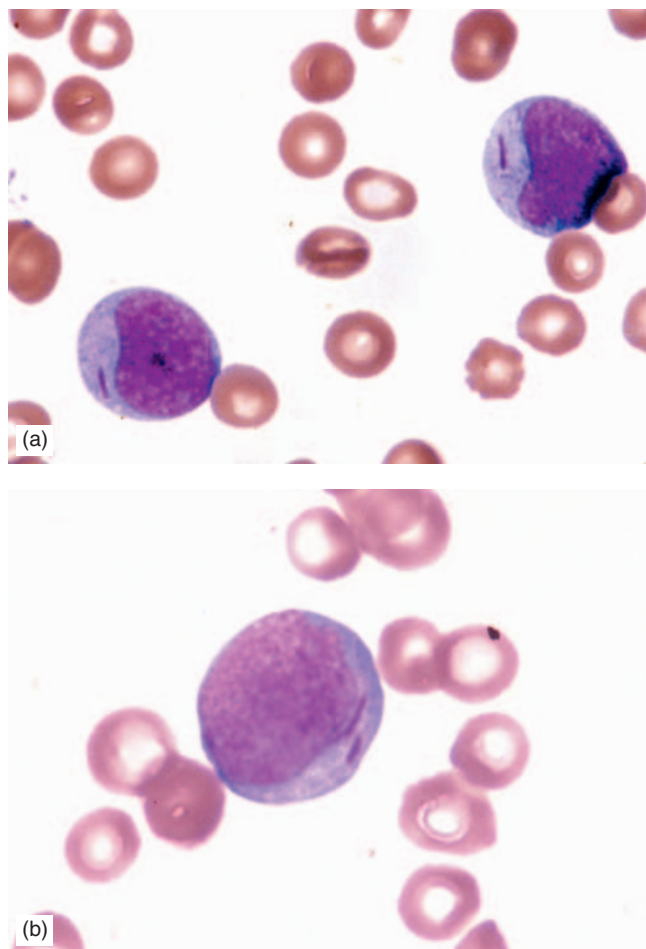


FIGURE 11.1 Peripheral blood smears (a and b) showing myeloblasts with Auer rods.

or promyelocytes. Erythroblasts are excluded from the blast count. In certain conditions, such as in the category of AML with recurrent genetic abnormalities, the requirement for $\geq 20\%$ blasts may be sidestepped. Three morphologic types of myeloblasts have been described: Types I, II, and III. Type I myeloblasts contain no cytoplasmic granules, type II myeloblasts contain $\geq 20\%$ cytoplasmic azurophilic granules, and type III myeloblasts contain 20 cytoplasmic azurophilic granules. Auer rods may be present (Figure 11.1). Myeloblasts are positive for CD13, CD33, and HLA-DR, and may express CD117, CD34, and myeloperoxidase (MPO). Promyelocytes are overall larger and carry larger quantities of azurophilic granules than myeloblasts. They depict a well-developed Golgi system and a round or an oval nucleus, which is often eccentric. Type III myeloblasts and promyelocytes share overlapping morphologic features, and therefore their distinction at times is difficult. Myeloblasts are HLA-DR-positive and may express CD34, whereas promyelocytes are negative for HLA-DR and CD34, but positive for CD13, CD33, and MPO and may express CD117. The morphologic features of monoblasts, promonocytes, erythroblasts, and megakaryoblasts are described later in the appropriate sections.

In general, the bone marrow biopsy/clot sections are hypercellular and show diffuse infiltration of the bone

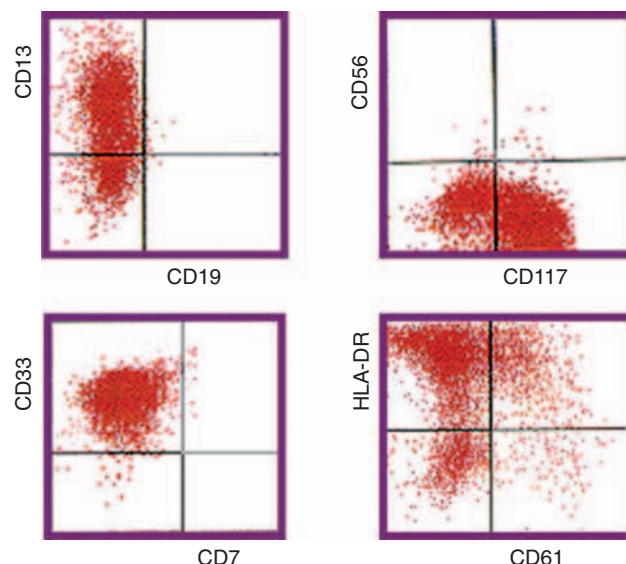


FIGURE 11.2 An example of dot plot analysis of an AML by flow cytometry. The blasts express myeloid-associated markers CD13, CD33, and CD117 and are positive for HLA-DR.

marrow by immature myeloid cells. Occasionally, the bone marrow is hypocellular. Blood examination may reveal anemia, leukopenia, and/or thrombocytopenia. Myeloid left shift is a common feature, and often a variable number of blasts are present. However, in some cases, at the time of bone marrow diagnosis, the peripheral blood smears may show no evidence of blast cells (aleukemic leukemia).

Immunophenotype and Cytochemical Stains

Immunophenotyping is an important component of bone marrow and blood evaluations in the current diagnostic work-up of acute leukemias for the following reasons [45, 46]:

1. Lineage assignment to distinguish AML from acute lymphoblastic leukemia (ALL) and to assign the leukemia to the proper subcategories, such as myeloblastic, monoblastic, erythroblastic, or megakaryoblastic (Figure 11.2).
2. Blast enumeration to confirm the presence of $\geq 20\%$ blast cells in the bone marrow or blood and to evaluate post-treated samples for residual disease.
3. Search for aberrant expressions of CD molecules to use for the detection of residual disease and possible prognostic values.
4. Detection of ambiguous (bilineal, biphenotypic) leukemias.

To fulfill the above-listed missions, the flow cytometry and immunohistochemical laboratories utilize a panel of monoclonal antibodies. This panel, which may vary considerably from one laboratory to another, should contain antibodies against CD molecules that help identify (1) blast hematopoietic cells, (2) cells of myeloid lineage, and (3) cells of lymphoid lineage. For example, the following panel is currently used for flow cytometric studies of

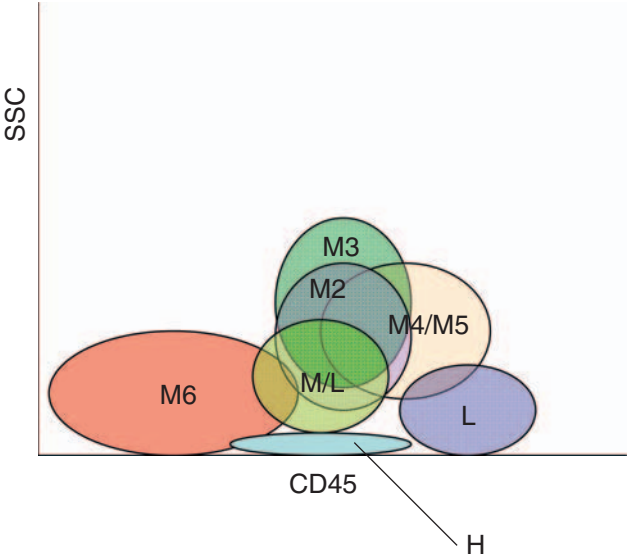


FIGURE 11.3 A diagram of SSC/CD45 features of hematopoietic malignancies by flow cytometry. H: hematogones, M/L: acute myeloid leukemias (minimally differentiated, without maturation) and acute lymphoid leukemias, L: mature lymphoid malignancies, M2: acute myeloid leukemia with maturation, M3: acute promyelocytic leukemia, M4/M5: acute myelomonocytic and acute monocytic leukemia, M6: erythroleukemia and multiple myeloma.

acute leukemia at the VA Greater Los Angeles Healthcare System:

Hematopoietic blasts	CD34, CD45, HLA-DR, and TdT
B-lineage	CD10, CD19, CD20, CD22, cytoplasmic CD22, and cytoplasmic CD79a
T-lineage	CD2, CD3, CD5, CD7, and cytoplasmic CD3
Myelomonocytic precursors	CD13, CD14, CD33, CD36, CD64, cytoplasmic CD13, and cytoplasmic MPO
Megakaryoblasts	CD41 and CD61
Erythroblasts	Glycophorin A (GPA) and CD71
Others	CD56, CD38

Four-color flow cytometry is the accepted standard of practice in most hematopathology laboratories. The side scatter (SSC)/CD45 characteristics of different types of hematopoietic malignancies are presented in Figure 11.3.

Most special cytochemical stains have been replaced by immunophenotyping in most laboratories. However, certain stains are still being used in the differential diagnosis and the classification of acute leukemia [47, 48].

Myeloperoxidase Stain: Myeloperoxidase is a lysosomal enzyme present in granulocytic and monocytic cells (Figure 11.4). MPO is expressed in neutrophilic and eosinophilic lineages in all stages of maturation, but in basophils it is more often detected in the immature forms. The mature basophils are usually negative for MPO. The intensity of MPO staining is less in monocytes than in granulocytes. Erythroid precursors and lymphocytes are MPO-negative. A peroxidase isoenzyme has been detected by electron

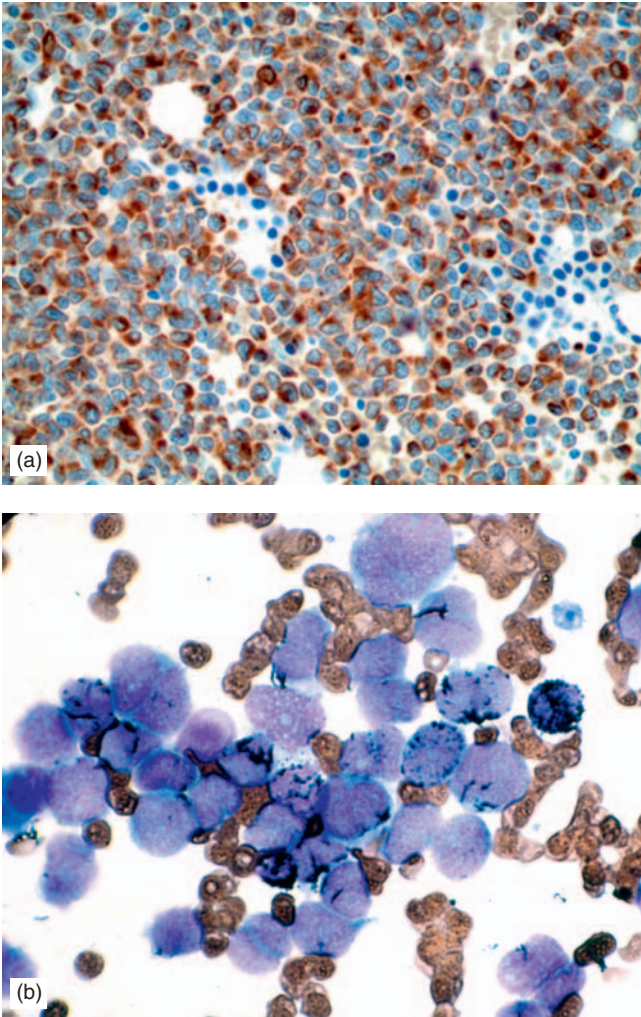


FIGURE 11.4 Immunohistochemical (a) and cytochemical (b) stains for MPO demonstrating numerous MPO-positive cells.

microscopy in the dense tubular system of platelets and megakaryocytes, but by conventional techniques, these cells are MPO-negative [49].

Myeloperoxidase activity declines rather rapidly. Air-dried unstained smears should be stored at cool temperatures, in the dark, and be used within 1–2 weeks.

Sudan Black B Stain: Sudan Black B is a lipophilic dye that stains the granulocytic series [47]. The pattern of reactivity of Sudan Black B in the granulocytic series is similar to that of MPO (Figure 11.5a). Monocytes are either negative or weakly positive with this stain. Lymphocytes, erythroid cells, megakaryocytes, and platelets are usually Sudan Black B-negative. Unlike MPO, Sudan Black B is stable, and therefore archival cytologic materials could be used for staining. Sudan Black B stain does not work in paraffin sections.

Periodic Acid-Schiff Reaction: Periodic acid-Schiff reaction in hematopoietic cells is primarily due to the presence of cytoplasmic glycogen. The granulocytic lineage and plasma cells show diffuse, fine PAS-positive granules, whereas dysplastic erythroid precursors (Figure 11.5b) and sometimes blasts in acute lymphoid leukemia, monocytic

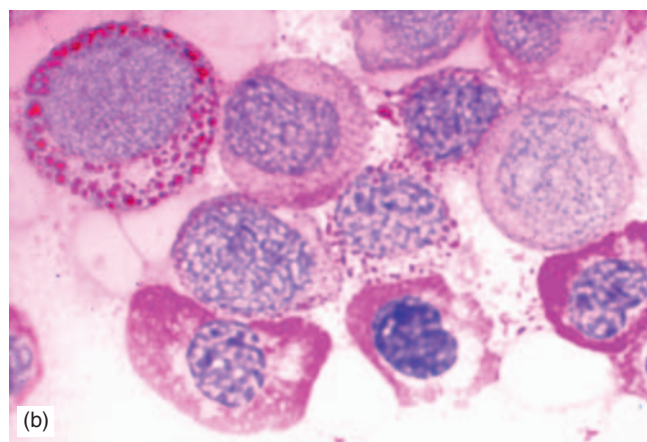
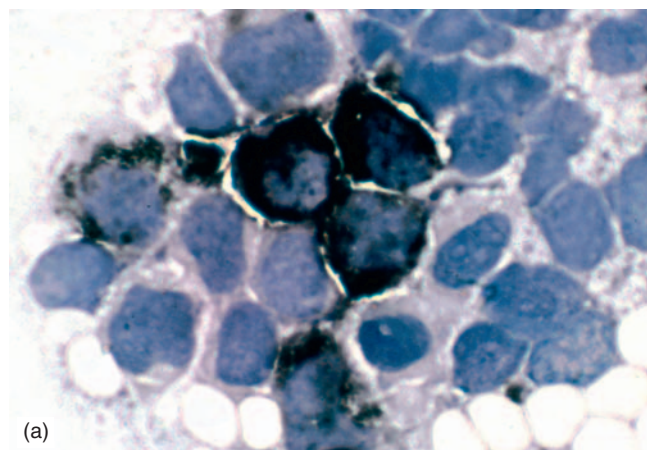


FIGURE 11.5 Bone marrow smears. (a) Cytochemical stains for Sudan Black B demonstrates dense, darkly stained cytoplasmic granules in myeloid cells. (b) Dysplastic erythroid precursors show coarse PAS-positive cytoplasmic granules.

leukemia, and megakaryocytic leukemia show coarse PAS-positive cytoplasmic granules.

Alpha-Naphthyl Butyrate Esterase: Alpha-naphthyl butyrate esterase, also known as *non-specific esterase* (NSE), is a monocytic marker (Figure 11.6a) [47]. This stain is helpful in distinguishing acute leukemias with monocytic differentiation, as well as histiocytic lesions. However, lymphoblasts, erythroblasts, and megakaryoblasts may also show a few cytoplasmic-positive granules. Granulocytic series are negative for an NSE stain. An NSE activity is fluoride sensitive.

Naphthol AS-D Acetate Esterase: Naphthol AS-D acetate esterase is demonstrated in all stages of maturation in the granulocytic and monocytic series [47]. Lymphoblasts, erythroblasts, and megakaryoblasts may also show a few punctuate cytoplasmic-positive granules. The enzyme activity is inhibited by sodium fluoride in monocytes but not in granulocytes.

Naphthol AS-D Chloroacetate: Naphthol AS-D chloroacetate is primarily expressed in the granulocytic series (Figure 11.6b) and mast cells [47]. Other hematopoietic elements are essentially negative, though some monocytes, megakaryocytes, lymphoid and erythroid cells and their leukemic

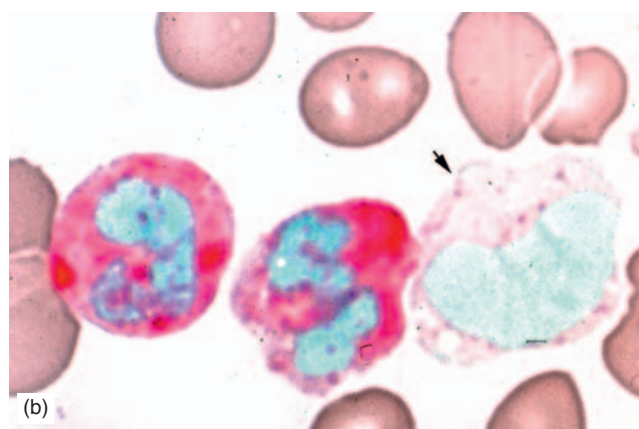
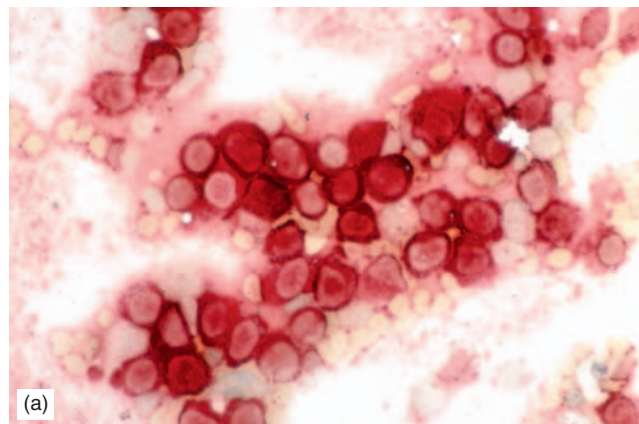


FIGURE 11.6 (a) Bone marrow smear showing numerous cells positive for an NSE stain. (b) Granulocytes stain for naphthol AS-D chloroacetate. A monocyte with a few cytoplasmic granules is present (arrow).

counterparts may show a weak reaction. Naphthol AS-D chloroacetate is very stable and is demonstrated in archival cytologic materials and paraffin-embedded tissue sections.

Molecular and Cytogenetic Studies

A garden variety of molecular genetic and cytogenetic abnormalities have been reported in AMLs. Some of these abnormalities are recurrent and more specific, such as $[t(8;21)(q22;q22);(RUNX1/RUNX1T1)]$, $[with\ t(15;17)(q11;q12);(PML/RAR\alpha)]$, $[inv(16)(p13q22)\ or\ t(16;16)(p13;q22);(CBF\beta/MYH11)]$, and $11q23\ (MLL)$ abnormalities, and others are non-specific [50–52]. The non-specific chromosomal abnormalities include translocations, trisomies, monosomies, deletions, and other structural changes which are described later with each AML subtype.

Clinical Aspects

The overall incidence of AML is about 3 per 100,000 population per year. The median age for AML onset is 60 years, with a male:female ratio of about 1 [53, 54]. The clinical symptoms are related to cytopenias and include weakness, fatigue, recurrent infections, and hemorrhagic episodes, such as gum bleeding or ecchymoses. Bone pain is infrequent.

Extramedullary infiltration (chloroma, granulocytic sarcoma) is occasionally seen, particularly in AMLs with monocytic differentiation. The most frequent extramedullary sites are skin, gum, and liver, but lymph nodes, intestinal tract, female reproductive systems, mediastinum, or other sites may also be involved. On rare occasions, the extramedullary involvement may be the very first presenting symptom.

Cytogenetic results are the most informative indicators of prognosis. The favorable karyotypes include t(8;21), t(15;17), and structural changes in 16q. Karyotypes with adverse clinical outcomes are monosomy 5 or 7, del(5q), and abnormal structural changes in 3q26. Resistant disease after first course of chemotherapy (>15% blasts in the bone marrow) also indicates poor prognosis. Five-year survival for the favorable prognostic category has been reported to be 70% with a 33% chance of relapse, whereas the figures for the poor prognostic category are 15% and 78%, respectively [55–57].

AML WITH RECURRENT GENETIC ABNORMALITIES

Several specific chromosomal translocations have been identified in AML patients, some of which are strongly associated with certain morphologic features. The most prominent translocations are t(8;21), t(15;17), t(16;16), or inv(16), and translocations involving the long arm of chromosome 11 (11q23).

A recently revised draft of the WHO classification has added several cytogenetic aberrations into this group, such as t(9;11)(p22;q23), t(1;22)(p13;q13), t(9;22)(q34;q11.2), and AML with mutation of *CEBPA* (new WHO classification in press [58b]). These entities are briefly discussed at the end of this chapter under Other Recurrent Genetic Abnormalities.

AML with t(8;21)(q22;q22);(*RUNX1/RUNXT1*)

Acute myeloid leukemia with t(8;21) is a relatively common leukemia accounting for about 18% of all cases of AML with cytogenetic abnormalities and 40% of AMLs with maturation (discussed later). In this balanced translocation, *RUNX1* (runt-related transcription factor 1), *AML1* gene, on the long arm of chromosome 21 (q22), fuses with the *RUNXT1* (acute myelogenous leukemia 1 translocation 1; also named MTG8, ETO or CBFA2T1) gene on the long arm of chromosome 8 (q22). This fusion results in an *RUNX1/RUNXT1* chimeric product.

Etiology and Pathogenesis

The etiology of AML with t(8;21) is not clear. Molecular studies suggest that altered transcriptional regulation and reduced apoptosis play important roles in the pathogenesis of this leukemia [58, 59]. The AML1 protein in conjunction with core-binding factor beta (CBF β) forms a transcription factor. The AML/CBF β transcription factor plays a regulatory role in a number of genes that are involved in myelogenesis and differentiation. Knockout of *AML1* or *CBF β* gene in mice leads to defective hematopoiesis and embryonic death.

Also, the *RUNX1/RUNXT1* fusion product reduces apoptosis by activating the expression of the anti-apoptosis gene *BCL-2* [60–62].

Pathology

Morphology

The morphologic features in a significant proportion of AML with t(8;21) are similar to those described in the category of AML with maturation (discussed later). Approximately 40% of AML with maturation show t(8;21) [1, 61, 63]. The myeloblasts are large, often with indented nuclei and basophilic cytoplasm. Type II and III myeloblasts are prominent and some blasts may contain large granules mimicking cytoplasmic granules seen in the Chediak–Higashi syndrome. Auer rods are frequent and also may be detected in the more mature myeloid forms. Promyelocytes, myelocytes, metamyelocytes, bands, and segmented neutrophils are present and often show dysplastic changes (Figure 11.7). Eosinophilia is common, and some cases may show increased bone marrow basophils and/or mast cells.

Approximately 7% of the AML cases with t(8;21) show morphologic features of acute myelomonocytic leukemia [64, 65] (discussed later). These patients depict peripheral blood monocytosis with the presence of immature forms and increased myeloblasts, monoblasts, and promonocytes in their bone marrows.

Rare cases of AML with t(8;21) may show blast counts of <20% in their blood or bone marrow.

Immunophenotype and Cytochemical Stains

Blasts and immature cells express myeloid-associated markers such as CD13, CD33, and CD117. CD34 is often positive and there may be aberrant expression of CD19 and/or CD56. Blast cells may show dim expression of TdT in a small proportion of cases. Because of the presence of type II and III myeloblasts and predominance of promyelocytes and myelocytes, the immature myeloid population on the CD45/SSC flow cytometry dot plot is moved up, demonstrating the granularity of the immature myeloid population. Blast cells are usually MPO- and/or NSE-positive.

Molecular and Cytogenetic Studies

The molecular detection of *RUNX1/RUNXT1* fusion is usually by fluorescence *in situ* hybridization (FISH) or reverse transcriptase polymerase chain reaction (RT-PCR) (Figure 11.8). It is important to know that some patients who have remained in continuous remission for a long period may still show *RUNX1/RUNXT1* mRNA in their leukocytes by RT-PCR techniques [43, 60, 67]. The clinical significance of the persistence of *RUNX1/RUNXT1* is not clear. However, the detection of *RUNX1/RUNXT1* fusion by itself may not indicate relapse or active disease.

The hallmark cytogenetic finding is the t(8;21)(q22;q22) (*RUNX1/RUNXT1*) (Figure 11.8). Complex translocations, such as t(8;21;14) or t(8;12;21), have been reported in occasional cases [68, 69]. Some patients may only show –Y or del(9q) [70]. In some cases, t(8;21) is cryptic and

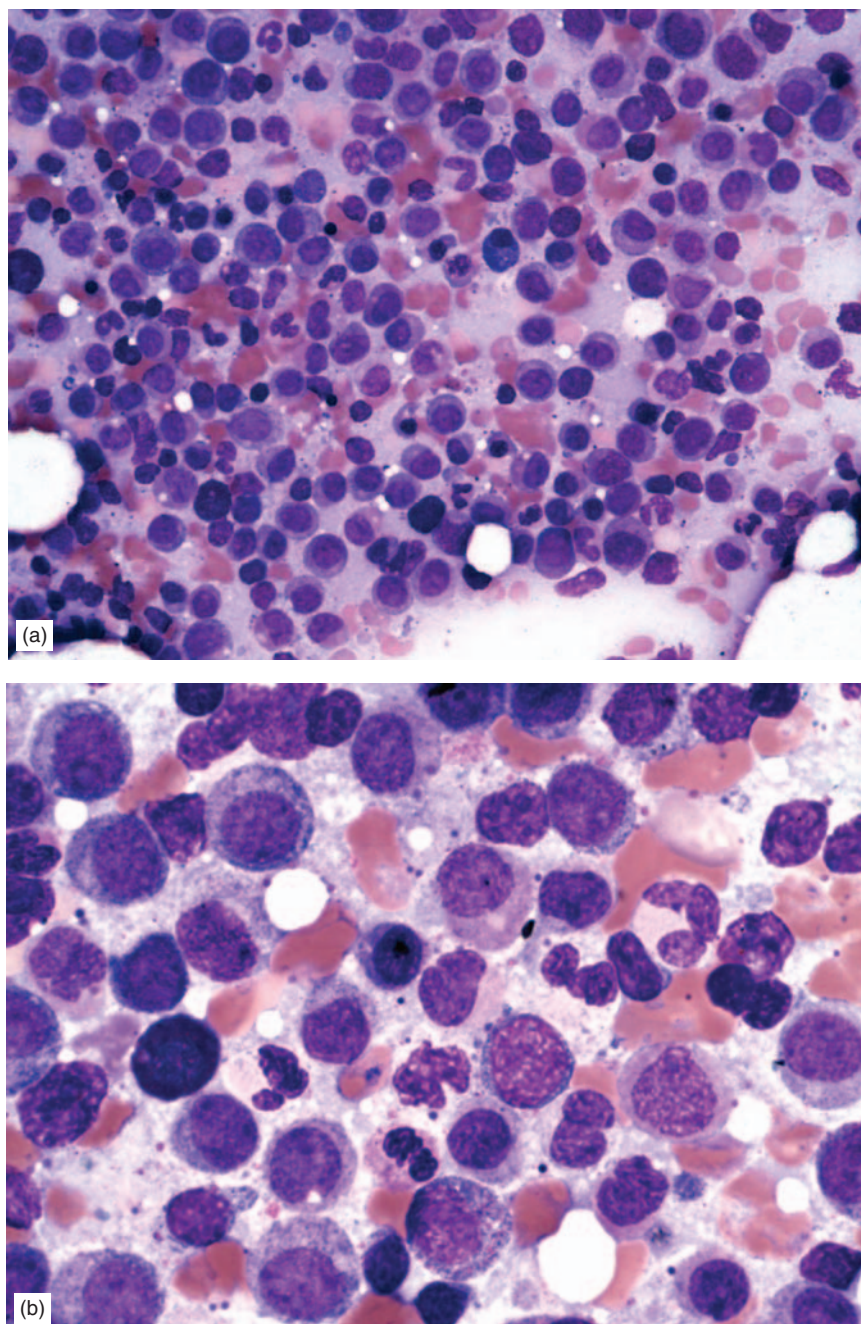


FIGURE 11.7 Bone marrow smear of a patient with t(8;21) AML demonstrating increased blasts with the presence of progressive maturation (acute myeloid leukemia with maturation, AML-M2): (a) low power and (b) high power.

undetectable by standard karyotyping. In such cases, FISH (Figure 11.8b) or RT-PCR technique is required to establish the *RUNX1/RUNXT1* rearrangement.

Clinical Aspects

Acute myeloid leukemia with t(8;21)(q22;q22) accounts for about 5–20% of AMLs in adults and is the most frequent AML in children [1, 60, 61]. The average age for adults is about 30 years, which is significantly lower than the average age for other types of AML. This leukemia has a

favorable prognosis in adults [59, 71, 72]. The clinical outcome in children is poor.

Acute Promyelocytic Leukemia

Acute promyelocytic leukemia (APL) is one of the variants of AML associated with t(15;17)(q11;q12);(*PML/RARα*) or other forms of chromosomal translocation involving the retinoic acid receptor- α (*RARα*) gene. APL accounts for 5–10% of all AMLs [1, 73, 74].

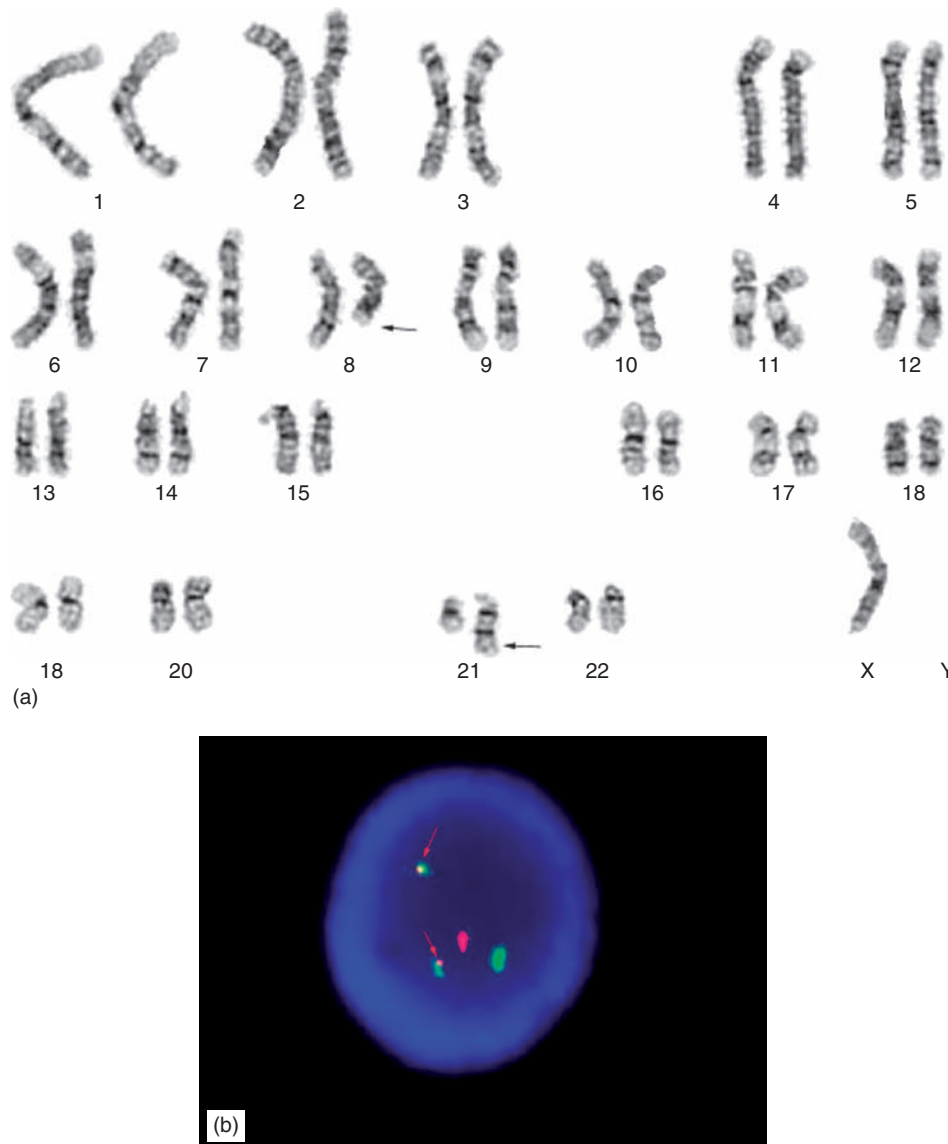


FIGURE 11.8 (a) G-banded karyotype showing t(8;21) (arrows). (b) The *RUNX1/RUNX1T1* fusion is demonstrated by FISH (arrows).

Etiology and Pathogenesis

The etiology of APL is not known. Alteration in the *RARα* gene appears to be the key pathobiologic event in the development of APL. *RARα* is primarily expressed in hematopoietic cells. It binds to retinoic acid to regulate the transcription of genes that are important in the differentiation pathway in hematopoiesis. The t(15;17) leads to the production of *PML-RARα* fusion protein which is less sensitive to retinoic acid. This reduced sensitivity to retinoic acid may lead to persistent transcriptional repression, and therefore, prevention of further differentiation of promyelocytes [74–76]. The *PML-RARα* fusion protein is also capable of blocking normal *RARα*-mediated functions. It has been shown that transgenic mice expressing *PML-RARα* fusion protein in the myeloid progenitors in their bone marrow eventually develop a promyelocytic-type leukemia [60, 74]. *In vitro* studies of human stem cells transfected by a *PML-RARα* cDNA containing a retroviral vector have shown

(1) a rapid induction of stem cell differentiation to promyelocytes, (2) maturation arrest at the promyelocyte stage, (3) preferential stem cell commitment to granulocytic differentiation, and (4) protection of apoptosis induced by the removal of hematopoietic growth factors [74, 77, 78]. The *PML* (promyelocytic leukemia) gene is suggested to encode a tumor suppressor protein essential for several signals in apoptosis and functions as a transcriptional co-activator with the *p53* tumor suppressor gene [74, 79, 80].

Pathology

Morphology

Two morphologic variants of APL have been described: APL with hypergranular promyelocytes and APL with microgranular (hypogranular) promyelocytes [1–4].

Hypergranular promyelocytes have a cytoplasm heavily loaded with azurophilic granules, which are often coarser

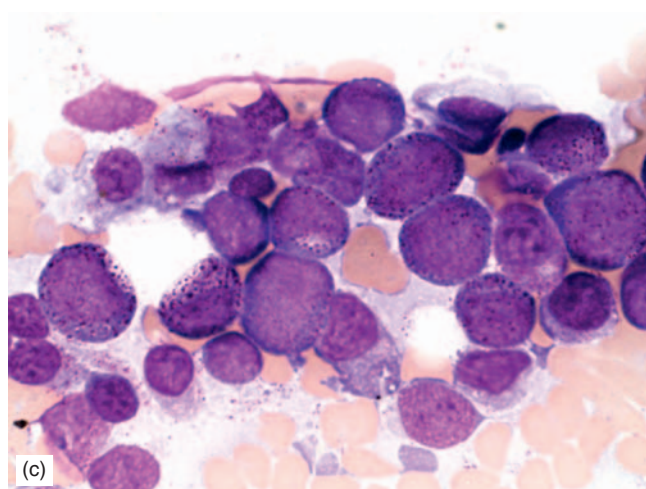
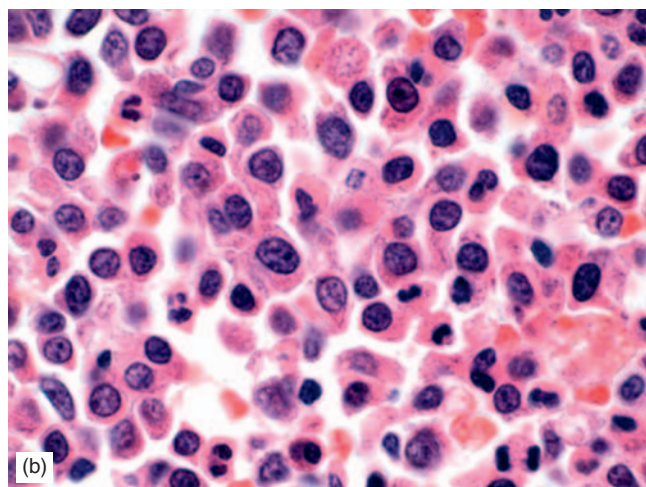
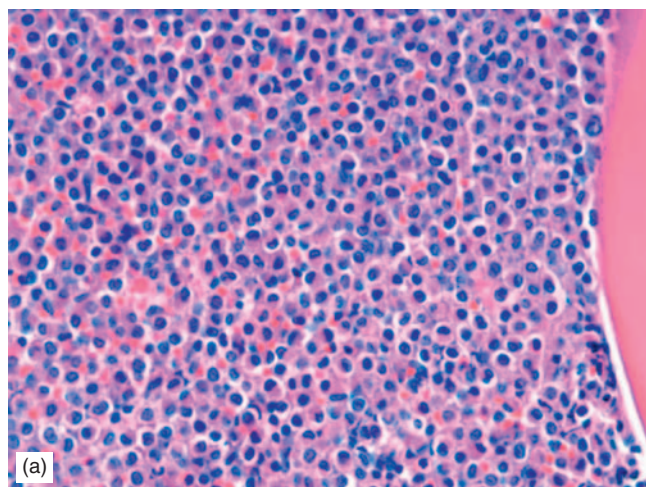


FIGURE 11.9 Acute promyelocytic leukemia. The bone marrow biopsy section shows sheets of immature cells with nuclear spacing and cytoplasmic granules (a and b). Numerous promyelocytes with numerous basophilic granules are demonstrated in the bone marrow smear (c).

and more numerous than the ones seen in normal promyelocytes (Figures 11.9 and 11.10). Auer rods are often present and in some cells appear in bundles (faggot cells) (Figures 11.10 and 11.11). Nuclei may appear round, irregular, folded, or dumbbell-shaped, but densely packed granules may obscure the visibility of the nuclei. The hypergranular promyelocytes are the predominant cells in the marrow, but smaller promyelocytes with basophilic cytoplasm and fewer azurophilic granules and microgranular promyelocytes are also present. Myeloblasts are less than promyelocytes and average around 10% of the bone marrow cells. The hypergranular variant, according to the literature, accounts for about 75–80% of the APLs. However, in our experience at the UCLA Medical Center and the VA Greater Los Angeles Healthcare System, we have seen more of microgranular variant than the hypergranular type.

In the remaining 20–25% of the cases of APL, the promyelocytes show abundant cytoplasm with lack of or sparse azurophilic granules. The azurophilic granules appear finer than the granules seen in the hypergranular variant (Figure 11.12). Auer rods and faggot cells may be present, but not so frequent as in the hypergranular subtype. The nuclei are predominantly bilobed, but folded and convoluted forms are often present, mimicking monocytic features. A small proportion of bone marrow cells may consist of myeloblasts, hypergranular promyelocytes, and small hyperbasophilic promyelocytes.

The bone marrow biopsy sections are hypercellular and show clusters and/or sheets of immature myeloid cells with abundant granular cytoplasm and nuclear spacing. Nuclei are commonly irregular or folded, and the nuclear chromatin is fine, often with prominent nucleoli.

The peripheral blood smears often show leukocytosis with the presence of atypical promyelocytes. A marked elevation of leukocyte count is seen more frequently in the microgranular variant.

Immunophenotype and Cytochemical Stains

The promyelocytes in APL show a homogenous expression of CD33 and often partial or dim expression of CD13 and CD15 (Figures 11.9–11.13). They are either negative for CD34 and CD117 or show partial expression. APL cells may also display aberrant expression of CD2, CD9, and/or CD56. The $t(11;17);(PLZF/RAR\alpha)$ variant is often CD13+ and CD56+, whereas the $t(5;17);(NPM/RAR\alpha)$ subtype is usually negative for CD13 and CD56 [81, 82]. HLA-DR and CD14 are commonly negative in APL cells. Promyelocytes are strongly MPO- and Sudan Black B-positive and -negative or weakly positive for NSE.

Molecular and Cytogenetic Studies

The genetic hallmark of APL is a translocation involving the $RAR\alpha$ gene. Four major translocations with the involvement of the $RAR\alpha$ gene have been associated with APL [74–76, 82–84]. These include:

- $t(15;17)(q23;q12);(PML;RAR\alpha)$
- $t(11;17)(q23;q12);(PLZF;RAR\alpha)$
- $t(11;17)(q23;q12);(NuMA;RAR\alpha)$
- $t(5;17)(q23;q12);(NPM;RAR\alpha)$

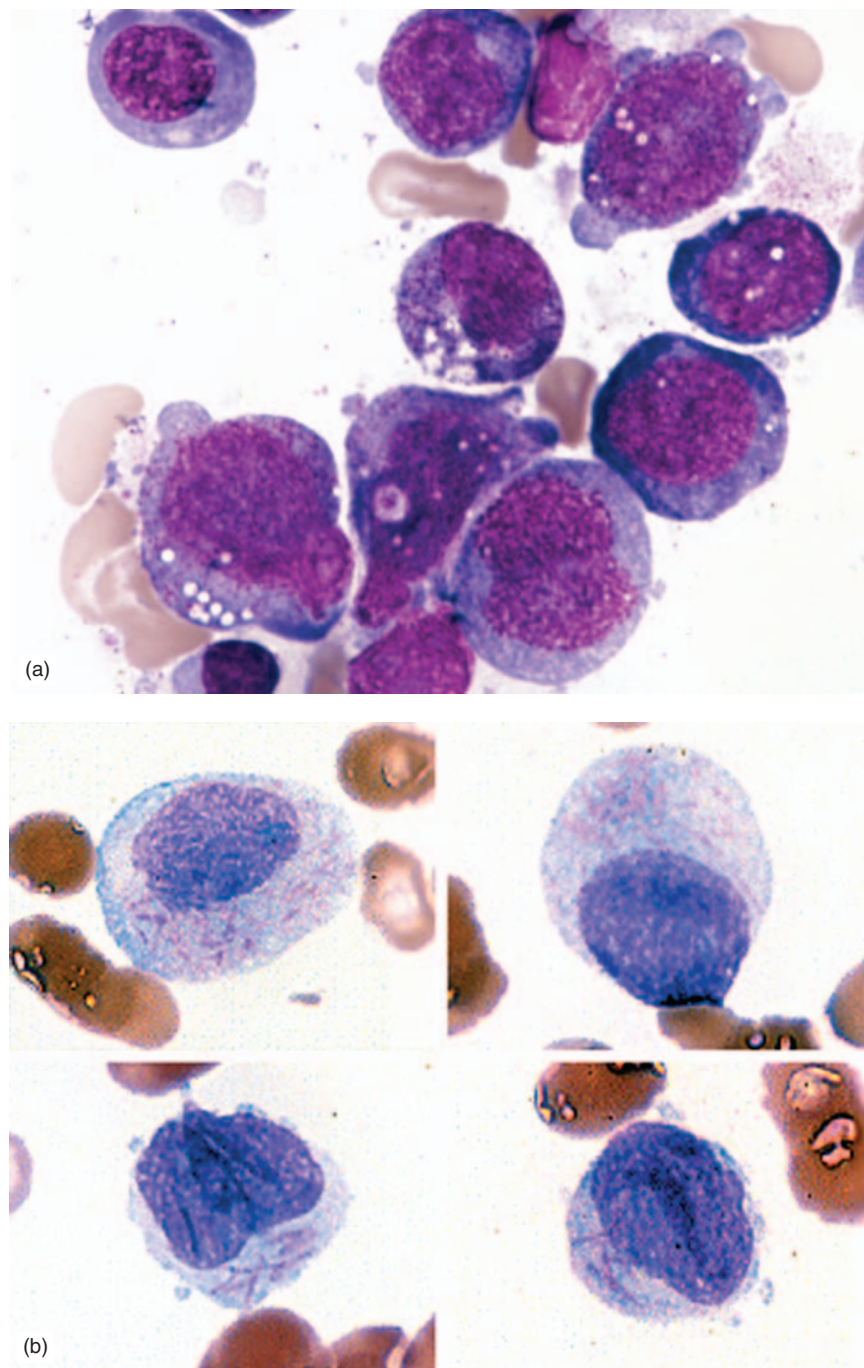


FIGURE 11.10 Acute promyelocytic leukemia. (a) Bone marrow smear demonstrating several dysplastic promyelocytes. (b) Promyelocytes with several Auer rods (faggot cells) are demonstrated (blood smears).

The most common translocation is $t(15;17)$ which is associated with the expression of $PML-RAR\alpha$ fusion protein (Figure 11.14a). The $t(11;17)(q23;q11.12)$ variant represents fusion of the $RAR\alpha$ gene with the $PLZF$ (promyelocytic leukemia zinc finger) gene (Figure 11.15). $PLZF$ protein is expressed in myeloid lineages and its expression is downregulated during differentiation. A rare variant, $t(11;17)(q13;q11.12)$, involves the $NuMA$ (nuclear matrix-mitotic apparatus protein) gene [84, 85]. Translocation of

$(5;17)(q35;q11.12)$ is another rare variant which involves the nucleophosmin (NPM) gene [74, 86]. This gene plays a role in the regulation of ribosomal nuclear processing and transport. Other infrequently reported genetic abnormalities include $STAT5b/RAR\alpha$ fusion, $der(7)(7;8)(q34;q21)$, $del(6p23)$, partial long arm deletion of chromosome 17, and complex four-way variant $t(15;17)$ [87–91].

FISH and RT-PCR studies (Figures 11.14b and 11.16) are routinely performed for the detection of $PML/RAR\alpha$

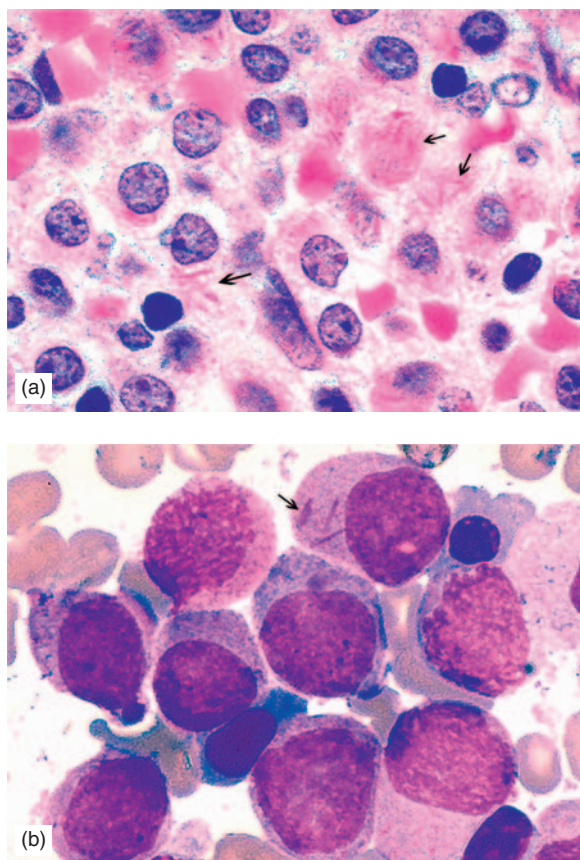


FIGURE 11.11 Bone marrow biopsy section (a) and smear (b) of a patient with acute promyelocytic leukemia showing abnormal promyelocytes with several Auer rods (faggot cells) (arrows).

fusion either to establish the diagnosis of promyelocytic leukemia or to rule out residual disease [86, 92, 93].

Clinical Aspects

Acute promyelocytic leukemia is primarily seen in young adults and middle-aged patients, but it may occur at any age. Clinical symptoms are related to complications of cytopenia and disseminated intravascular coagulopathy (DIC) [84, 94]. Weakness/fatigue, infection, and hemorrhagic episodes are often complications of anemia, granulocytopenia, and thrombocytopenia, respectively. DIC is either present at diagnosis or detected soon after chemotherapy. DIC is a serious complication which may lead to cerebrovascular or pulmonary hemorrhage in up to 40% of patients. The risk is reported to be higher in the microgranular variant of APL. Three major factors may contribute to the mechanism of DIC: (1) release of tissue factor which is involved in the activation of factor X through factor VII, (2) release of cancer procoagulants which activate factor X independent of factor VII, and (3) increased expression of annexin II receptor on leukemic promyelocytes [94, 95]. Annexin II receptor binds plasminogen and increases plasmin formation [95].

Acute promyelocytic leukemia is one of the favorable types of AML. Favorable prognostic factors include age under 30 years, initial leukocyte count $<10,000/\mu\text{L}$, and

platelet count $>40,000/\mu\text{L}$ [94, 96]. There are studies suggesting that the expression of CD56 on the leukemic promyelocytes, methylation of *p15* kinase inhibitor gene, and $t(11;17);(PLZF/RAR\alpha)$ are associated with less favorable prognosis [94, 96, 97].

All-*trans* retinoic acid (ATRA) is a highly effective therapeutic agent [98–100]. It accelerates the terminal differentiation of leukemic promyelocytes and induces clinical remission. For complete molecular remission and long-term survival, a combination of ATRA and cytotoxic chemotherapy is necessary. APL patients with $t(11;17);(PLZF/RAR\alpha)$ do not respond to ATRA.

AML with $inv(16)(p13q22)$ or $t(16;16)(p13;q22);(CBF\beta/MYH11)$

Acute myeloid leukemia with $inv(16)(p13q22)$ or $t(16;16)(p13;q22)$ is one of the variants of AML characterized by fusion of the *CBFβ/MYH11* genes. This leukemia depicts myelomonocytic differentiation with the presence of abnormal eosinophils. It accounts for about 10% of all AMLs [1].

Etiology and Pathogenesis

The etiology of AML with structural abnormalities of chromosome 16 and *CBFβ/MYH11* fusion is not known. It has been suggested that the *CBFβ/MYH11* (core-binding factor, beta subunit/smooth muscle myosin heavy chain 11) fusion protein inhibits the function of the *AML1/CBFβ* transcription factor leading to the repression of transcription [101–103]. The *CBFβ/MYH11* fusion gene disrupts the normal transcription factor activity of CBF functions as a class II mutation. In addition, most of these patients are known to possess mutually exclusive mutations of the receptor tyrosine kinases (RTKs), *c-KIT*, and *FLT3*, as well as *RAS* genes. These sets of mutations provide a paradigm for the “two-hit” hypothesis of leukemogenesis [103].

Pathology

Morphology

The bone marrow samples show myeloid left shift, increased number of immature myelomonocytic cells, and the presence of atypical eosinophilic precursors (Figures 11.17 and 11.18). Myeloblasts (including types II and III), monoblasts, and promonocytes usually account for $\geq 20\%$ of the total marrow cells, but occasionally may be less. Eosinophils usually constitute $>5\%$ of the marrow differential counts and appear to be a part of the leukemic clone. Some of the eosinophilic promyelocytes, myelocytes, and metamyelocytes contain large purple-violet granules in addition to eosinophilic granules [1–4]. These atypical granules are rarely found in more mature eosinophils. In rare cases of AML with $inv(16)(p13q22)$ or $t(16;16)(p13;q22)$, the atypical eosinophils may not be present, or instead of both myeloid and monocytic differentiation, the acute leukemia may represent only myeloid or only monocytic features.

The bone marrow biopsy and clot sections are usually hypercellular with increased immature myeloid forms and increased blasts. There is often evidence of eosinophilia.

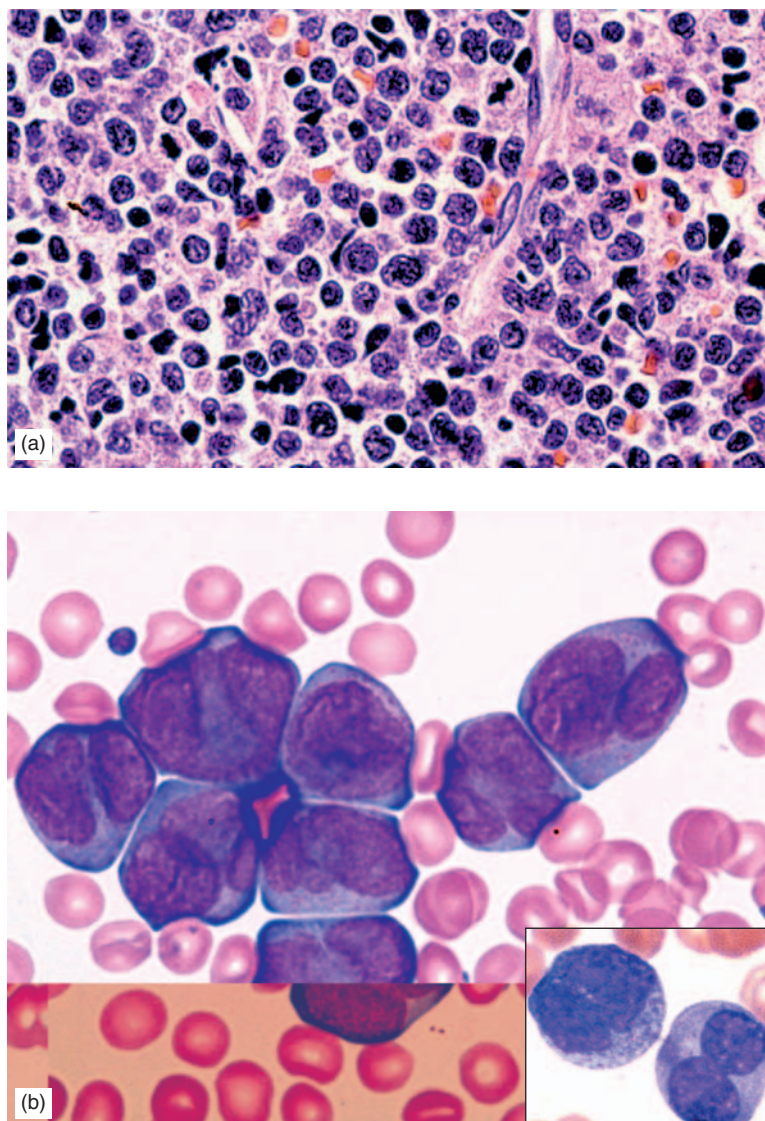


FIGURE 11.12 Acute promyelocytic leukemia, hypogranular variant. Bone marrow biopsy section (a), bone marrow smear (b), and peripheral blood smear (inset) showing hypogranular promyelocytes with convoluted nuclei.

The blood smears may show eosinophilia with the presence of blasts and promonocytes. Absolute monocytosis is a frequent finding.

Immunophenotype and Cytochemical Stains

The immature myelomonocytic population expresses CD13 and CD33 with a partial expression of CD11c, CD14, CD15, CD34, CD36, CD64, CD117, HLA-DR, and MPO by flow cytometry. Immunohistochemical stains such as MPO, lysozyme, and CD68 are used for the evaluation of the bone marrow myelomonocytic component, and CD34 and CD117 stains are often helpful for the estimation of blast cells. An aberrant expression of CD2 has been frequently observed.

The cytochemical stains show strong MPO positivity for the granulocytic lineage and various degrees of diffuse cytoplasmic NSE staining for the monocytic population. The abnormal eosinophils may be weakly positive for naphthol AS-D chloroacetate esterase.

Molecular and Cytogenetic Studies

CBF β /MYH11 fusion with *inv(16)(p13q22)* or *t(16;16)(p13;q22)* is the characteristic genetic feature of this leukemia (Figures 11.19 and 11.20) [1, 103, 104]. The fusion transcript is detected by RT-PCR. Karyotyping and FISH studies reveal chromosome 16 paracentric inversion or translocation between the two chromosome 16s. Other associated cytogenetic abnormalities include trisomies 8, 21, and 22 as well as the loss of Y-chromosome deletion of the long arm of chromosome Y [104–106].

Clinical Aspects

Acute myeloid leukemia with structural abnormalities of chromosome 16 and *CBF β /MYH11* fusion mostly occur in middle-aged patients, but it may occur at any age. The leukemia is associated with a favorable prognosis with a complete remission rate of >90%. In a large retrospective

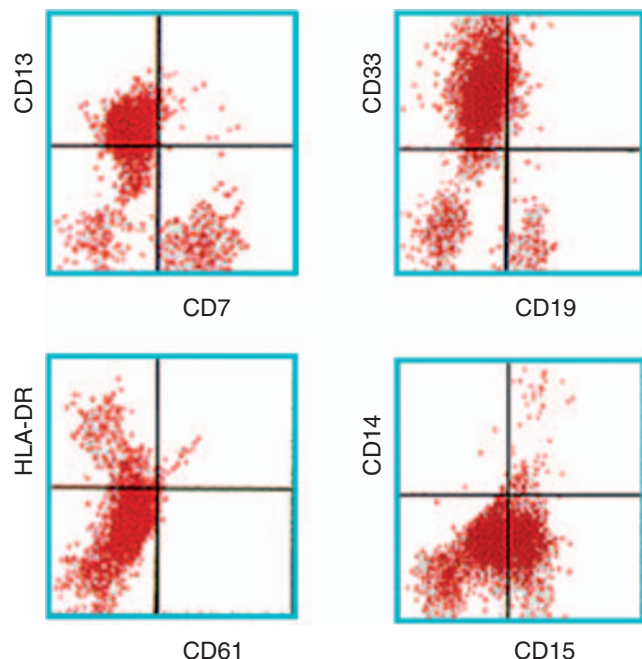


FIGURE 11.13 Acute promyelocytic leukemia. Flow cytometric studies reveal expression of CD13, CD15, and CD33 in a large population of cells. Only a small number of cells are positive for HLA-DR or CD14.

clinical survey of 110 patients conducted by the French AML Intergroup, the median age at diagnosis was 34 years with a female:male ratio of slightly >1 [104]. The estimated overall survival at 3 years was 58%. The adverse prognostic factors include age >35 , elevated WBC of $\geq 120,000/\mu\text{L}$ and thrombocytopenia of $\leq 30,000/\mu\text{L}$. A combination of cytotoxic drugs, such as daunorubicin and cytarabine with or without mitoxantrone, has been used for induction therapy.

AML with 11q23 (*MLL*) Abnormalities

The 11q23 (*MLL*) abnormalities are frequently observed in AMLs with monocytic differentiation, such as acute myelomonocytic and acute monocytic leukemias.

Over 73 different recurring translocations involving more than 50 partner genes have been reported involving 11q23 in acute leukemias [60, 94, 107].

Etiology and Pathogenesis

The etiology of AML with 11q23 abnormalities is not known. These abnormalities involve the *MLL* (mixed-lineage leukemia) gene with translocation breakpoints in between exons 5 and 11. The *MLL* protein has a potential DNA-binding site (AT-hook) which is able to bind to DNA and regulate the expression of genes that are important in hematopoiesis, including the development of myelomonocytic lineages [60, 94]. The translocation of the *MLL* gene results in a chimeric gene product that may play a role in leukemogenesis.

Chimeric mice with $t(9;11);(AML/AF9)$ have been shown to develop AML [108].

Pathology

Morphology

Monocytic differentiation is one of the hallmarks of AMLs with 11q23 abnormalities. Most of the cases fall into the category of acute myelomonocytic or acute monocytic leukemia with increased numbers of monoblasts and promonocytes in the bone marrow or peripheral blood (Figure 11.21) (discussed later). Monoblasts have variable amounts of dark-blue cytoplasm with no or few azurophilic granules or vacuoles. The nuclei are round or slightly indented or folded. The nuclear chromatin is finely dispersed and one or more prominent nucleoli are present. Promonocytes have more abundant cytoplasm which is less basophilic. They may contain few cytoplasmic azurophilic granules or vacuoles. The nuclei are irregular, folded, or convoluted with fine nuclear chromatin and often inconspicuous nucleoli.

Immunophenotype and Cytochemical Stains

The monoblasts and promonocytes express CD4 (dim), CD11c, and CD14. CD34 and CD117 are usually negative. Myeloblasts often express CD13, CD33, and CD117 and may express CD34. CD36, CD64, and HLA-DR are usually expressed on both myeloblasts and monocytic precursors.

Immunohistochemical stains such as MPO, lysozyme, and CD68 are used for the evaluation of the bone marrow myelomonocytic component, and CD34 and CD117 stains are often helpful in the estimation of myeloblasts.

The cytochemical stains show strong MPO positivity for the granulocytic lineage and various degrees of diffuse cytoplasmic NSE staining for the monocytic population.

Molecular and Cytogenetic Studies

The gene involved in the translocation of 11q23 is the *MLL* (also known as *ALL1* or *HRX*) gene [60, 109, 110]. Over 73 different translocations involving the *MLL* gene have been reported in both acute myeloid and lymphoid leukemias [60, 107, 111, 112–116]. The rearrangement of 11q23 is common in patients with acute myelomonocytic and acute monocytic leukemias, particularly in children [60, 117–119]. Approximately 75% of acute leukemias in infants under 1 year show 11q23 abnormalities [110, 120, 121]. These leukemias are of acute lymphoblastic type or AML with monocytic differentiation. The translocation of $(4;11)(q21;q23);(AF4;MLL)$ is the common translocation in children with pre-B ALL [122], and $t(9;11)(p22;q23);(MLLT3;MLL)$ or $t(10;11)(p12;q23);(ABI1;MLL)$ have been reported in association with adult myeloid leukemias [113, 114].

Clinical Aspects

Myeloid leukemias with abnormalities of 11q23 account for about 5% of total AMLs. In infants younger than



FIGURE 11.14 (a) G-banded karyotype showing t(15;17) (arrows). (b) The *PML-RAR α* fusion is demonstrated by a single fusion PML (red) and *RAR α* (green) FISH probe (arrow).

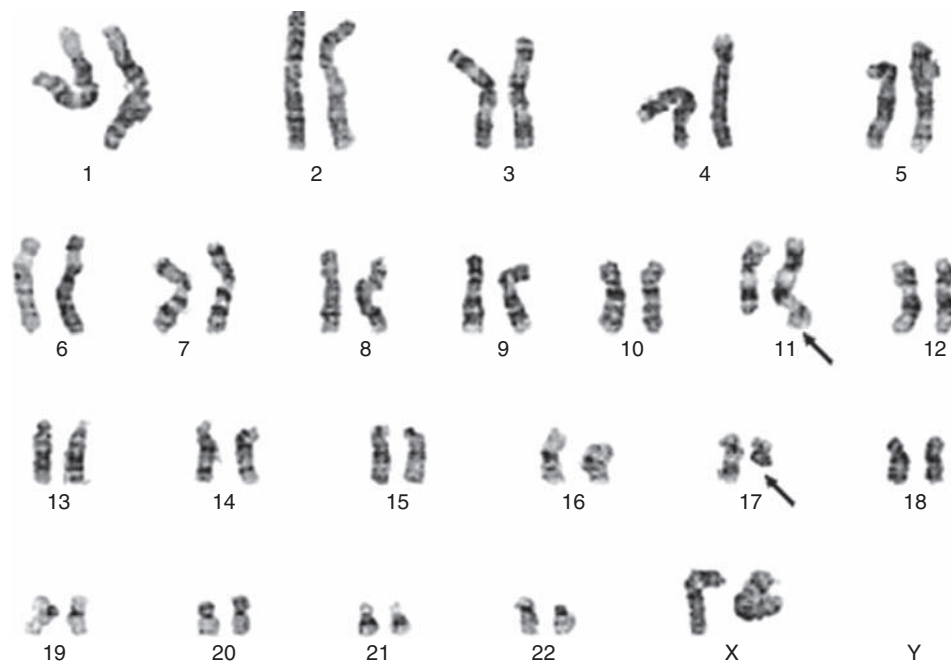
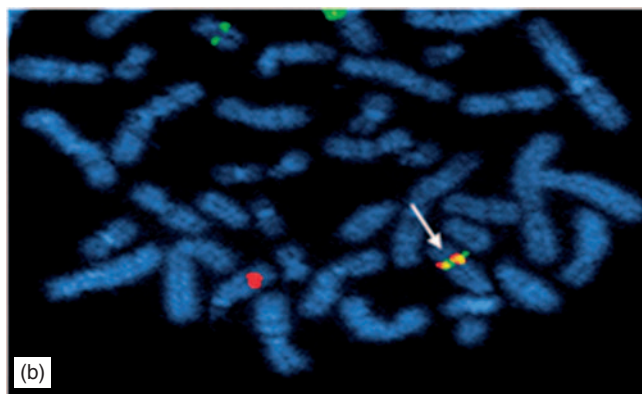


FIGURE 11.15 G-banded karyotype showing t(11;17) (arrows).

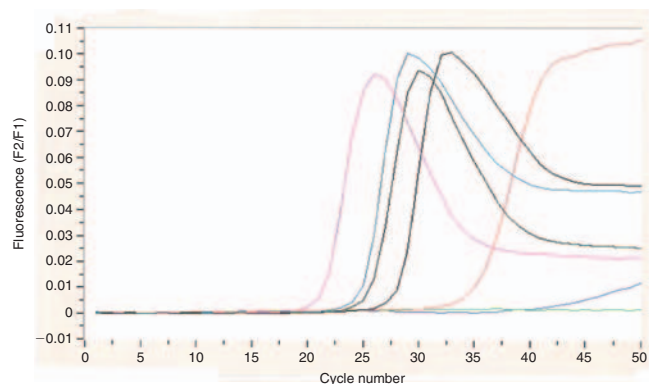


FIGURE 11.16 RT-PCR technique demonstrating a positive signal (the blue curve) in a patient with *PML/RARα* fusion (acute promyelocytic leukemia). Other curves are positive controls. The flat green and blue lines are negative controls.

1 year, up to 75% of all acute leukemias involve translocation of 11q23. These leukemias are primarily of acute myelomonocytic, acute monocytic, or acute lymphoid types. The translocation of 11q23 in adults is often associated with therapy-related leukemias, particularly after treatment with topoisomerase II inhibitors (discussed later). The leukemias with 11q23 abnormalities appear to fall into the category of leukemias with intermediate survival rate.

Differential Diagnosis

Cytogenetic aberrations are the main pathognomonic markers in AMLs with recurrent genetic abnormalities. However, certain morphologic and immunophenotypic features are helpful in distinguishing these leukemias. For example, a significant proportion of AMLs with t(8;21) are similar to those described in the category of AML with maturation, with the presence of type II and III myeloblasts and predominance of promyelocytes and myelocytes (discussed later).

Acute promyelocytic leukemia, particularly the microgranular variant, may mimic acute leukemias with monocytic differentiation. The leukemic promyelocytes, unlike monocytic cells, often show several Auer rods, are strongly MPO and Sudan Black B-positive, and do not express CD4, CD14, or HLA-DR (Table 11.2).

Acute myeloid leukemia with inv(16)(p13q22) or t(16;16)(p13;q22) depicts myelomonocytic differentiation with the presence of abnormal eosinophils. Most of the acute leukemias with 11q23 (*MLL*) abnormalities are morphologically of myelomonocytic or monocytic types.

AML WITH MULTILINEAGE DYSPLASIA

Acute myeloid leukemia with multilineage dysplasia is defined in the WHO classification as an acute leukemia with the presence of $\geq 20\%$ myeloid blasts in bone marrow or blood and the evidence of multilineage dysplasia [1]. Dysplastic changes occur in more than one lineage with ≥ 50 cells in each lineage affected. Megakaryocytic dysplasia is a

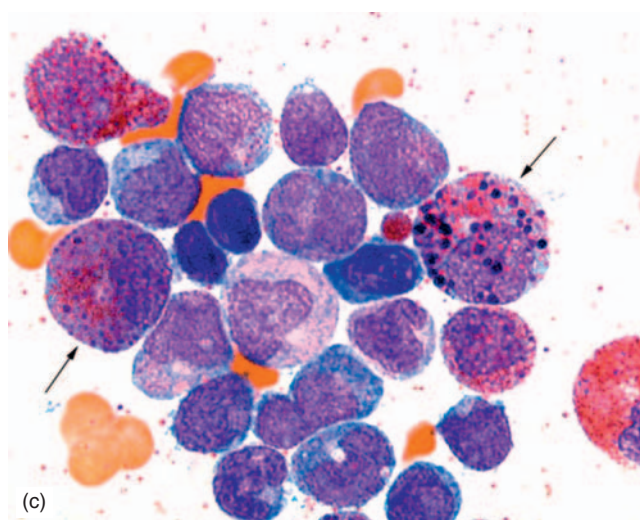
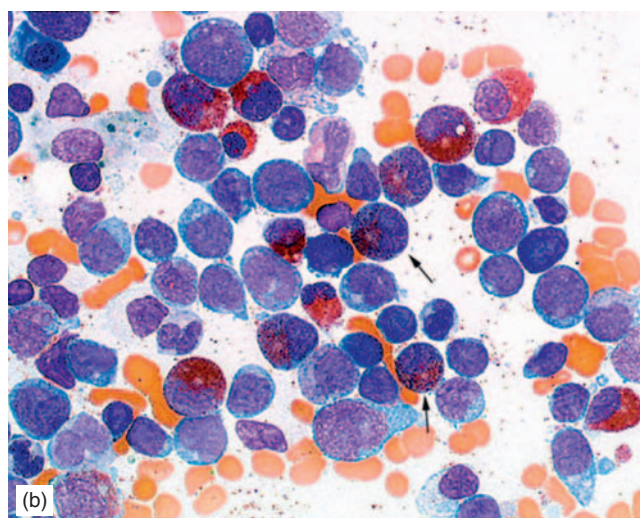
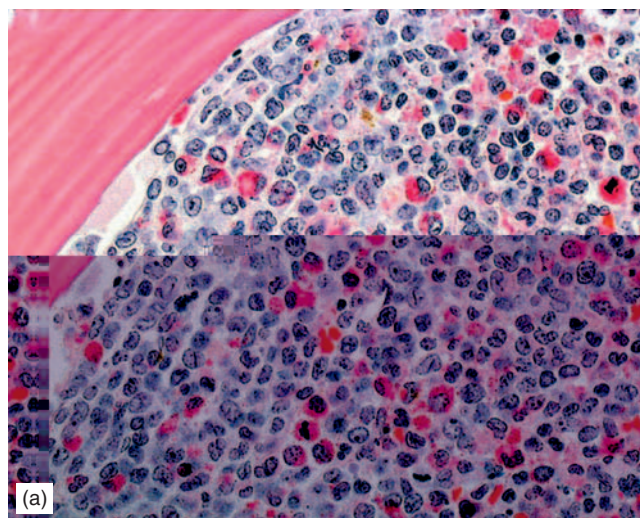


FIGURE 11.17 Bone marrow biopsy section (a) and bone marrow smear (b and c) from a patient with acute myelomonocytic leukemia with atypical eosinophilia and inv(16)(p13q22). Atypical eosinophils contain a mixture of eosinophilic and basophilic granules (arrows).

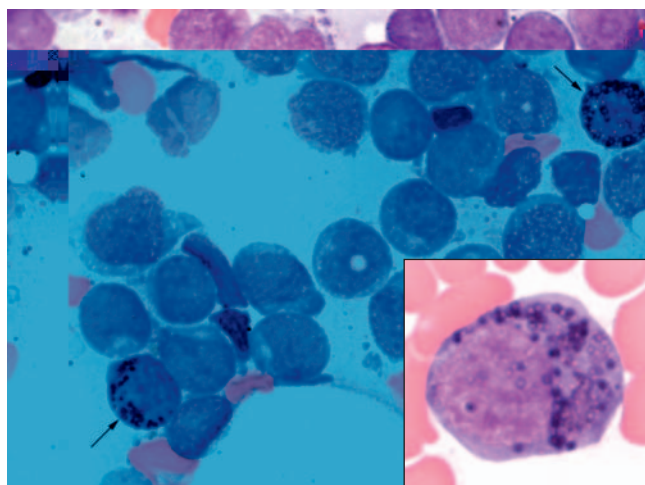


FIGURE 11.18 Bone marrow smear from a patient with acute myelomonocytic leukemia with atypical eosinophilia and *inv(16)(p13q22)*. Atypical eosinophils contain a mixture of eosinophilic and basophilic granules (arrows and inset).

dominant feature. Dysplastic changes may precede the development of acute leukemia but remain as a part of the picture.

Etiology and Pathogenesis

A significant proportion of patients with AML and multilineage dysplasia follow a history of MDS, and therefore share the etiology and pathogenesis of MDS (see Chapter 8).

Pathology

Morphology

The presence of $\geq 20\%$ blasts and significant multilineage dysplasia are the diagnostic hallmarks for this category. Dysplastic changes may involve all non-lymphoid hematopoietic cells, but often affect megakaryocytic and myeloid lineages (Figure 11.22). At least 50% of the cells in each lineage must show dysplastic morphology [1, 123].

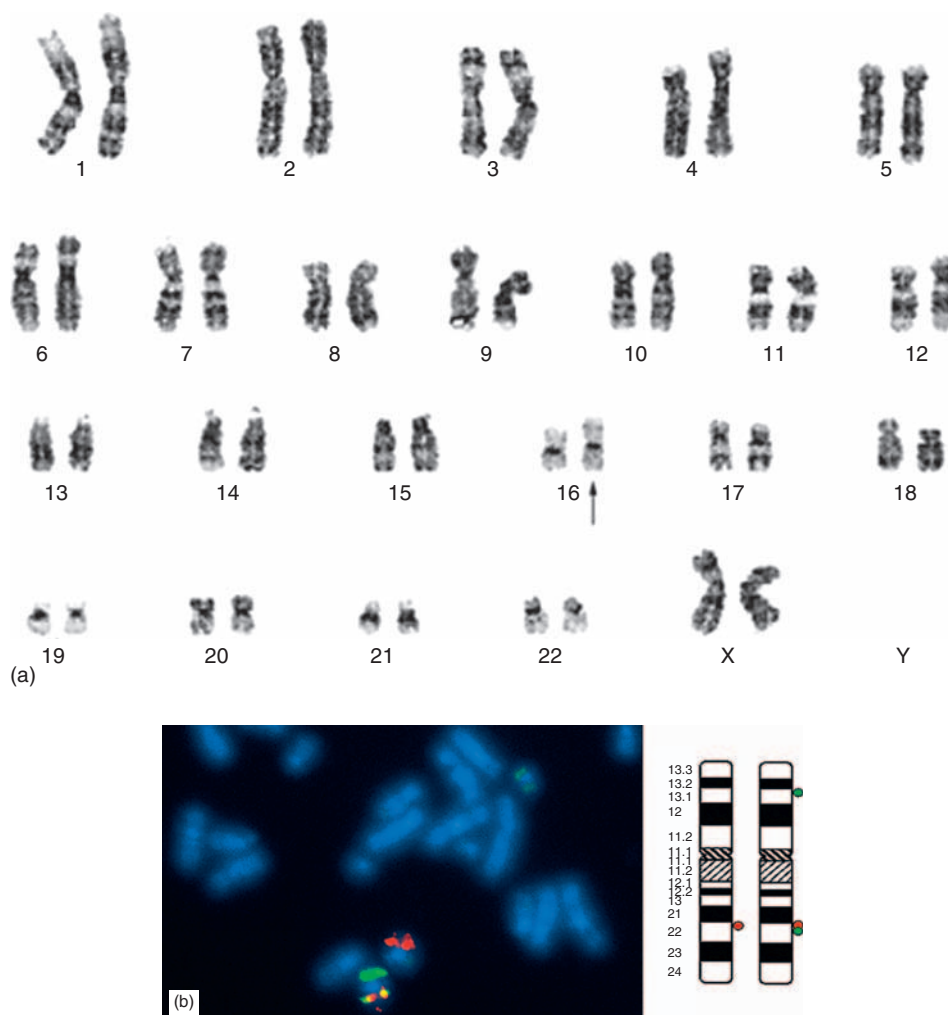


FIGURE 11.19 A *t(16;16)* is demonstrated by karyotyping (a) and FISH analysis (b) in a patient with acute myelomonocytic leukemia with atypical eosinophilia.

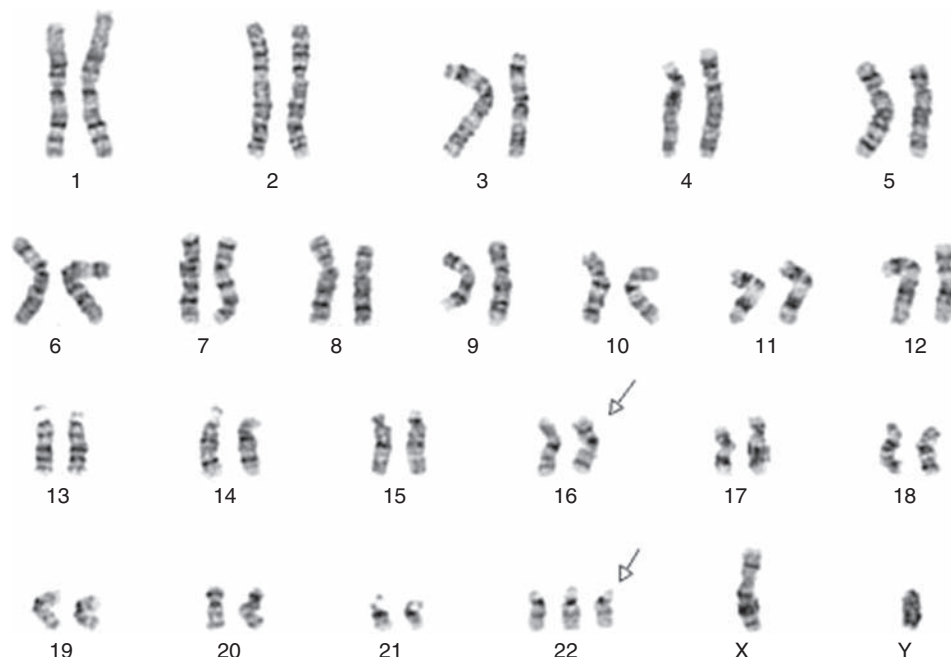


FIGURE 11.20 Karyotyping of bone marrow cells in a patient with acute myelomonocytic leukemia demonstrating inv(16)(p13q22) and trisomy 22.

Micromegakaryocytes with hypogranular cytoplasm, hypolobated nuclei, and/or mono- or binucleated forms are prominent. Large bizarre megakaryocytes may also be present. Neutrophilic series are often hypogranular and hyposegmented or may show abnormal segmentation. Erythropoiesis may appear megaloblastic or show nuclear budding or fragmentation. Ringed sideroblasts may be present.

Pancytopenia is a frequent finding in blood examinations. Blasts are often present in various numbers and dysplastic changes are observed, and these may be more obvious in the peripheral blood smears than the bone marrow smears. Biopsy and clot sections are usually hypercellular and show myeloid left shift with increased blasts.

Immunophenotype

Flow cytometry shows a population of blast cells expressing myeloid-associated markers. CD34 and CD117 are frequently expressed. In some cases, blasts may show aberrant expression of CD4, CD7, and/or CD56. The hypogranularity of the myeloid cells may lead to a lower SSC in flow cytometric dot plot preparations. The myeloid precursors may show increased expression of CD11a and CD66 and reduced expression of CD10 and CD116.

Immunohistochemistry may help to estimate the blast component and myeloid proportion by using CD34, CD117, and MPO stains.

Molecular and Cytogenetic Studies

No recurrent molecular or cytogenetic changes have been reported in this group. A garden variety of chromosomal deletions, monosomies, and trisomies have been observed, such as $-5/\text{del}(5q)$, $-7/\text{del}(7q)$, $\text{del}(11q)$ (Figure 11.23), $\text{del}(12p)$, $\text{del}(20)$ (Figure 11.25), and trisomy 8, 9, 11, 18, 19, and 21. Most of these changes are also frequently associated with MDS and chronic myeloproliferative disorders

(see Chapters 8 and 9). Non-specific translocations, such as $\text{t}(1;7)(q10;p10)$, $\text{t}(3;21)(q26;q22)$, and $\text{t}(6;9)(p23;q34)$, have also been occasionally observed [123–127]. The possibility of distinguishing AML with multilineage dysplasia from other subtypes based on gene-expression profiling has been suggested [128].

Clinical Aspects

Acute myeloid leukemia with multilineage dysplasia is a disease of the elderly with a median age of 60 years [53, 54]. Cytogenetic risks, age, and multilineage dysplasia have been reported to correlate inversely with the overall survival in the AML patients [129–131].

Differential Diagnosis

Acute myeloid leukemia with multilineage dysplasia is distinguished from refractory anemia with excess blasts (RAEB) by the presence of $\geq 20\%$ blasts in bone marrow and/or peripheral blood. It has morphologic overlapping features with acute erythroid leukemia (erythroid/myeloid), but unlike erythroid leukemia, in this leukemia the bone marrow erythroid component is not $\geq 50\%$. The requirement of significant multilineage dysplasia of $>50\%$ of the affected cells distinguishes this leukemia from most other types of AML.

AML AND MDS, THERAPY RELATED

Therapy-related AML (t-AML) and therapy-related MDS (t-MDS) represent spectrums of a progressive clonal hematopoietic disorder which is evolved following cytotoxic chemotherapy and/or irradiation [1, 132–134]. The

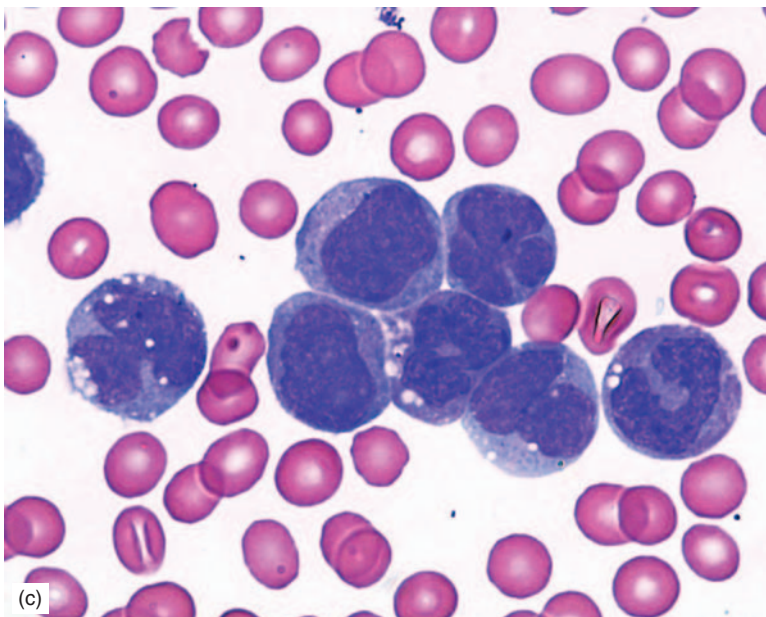
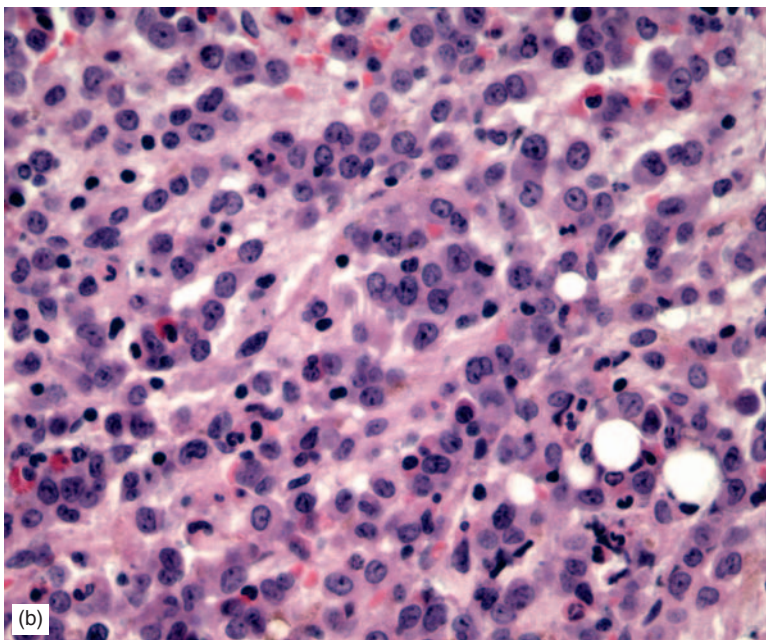
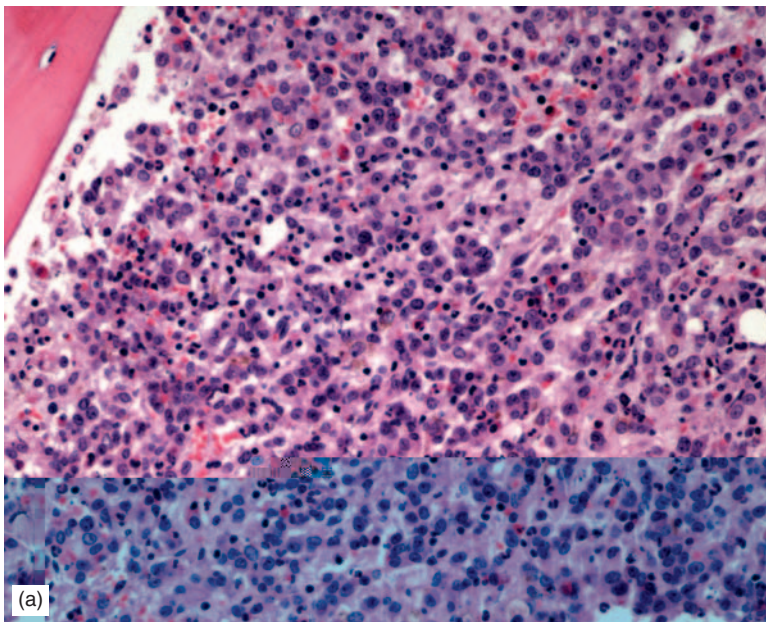


FIGURE 11.21 Acute monocytic leukemia in a patient with 11q23 abnormality. Biopsy section (a: low power and b: high power) reveals numerous immature cells with convoluted nuclei and fine nuclear chromatin. Peripheral blood smear (c) demonstrates abnormal promonocytes and monocytes.

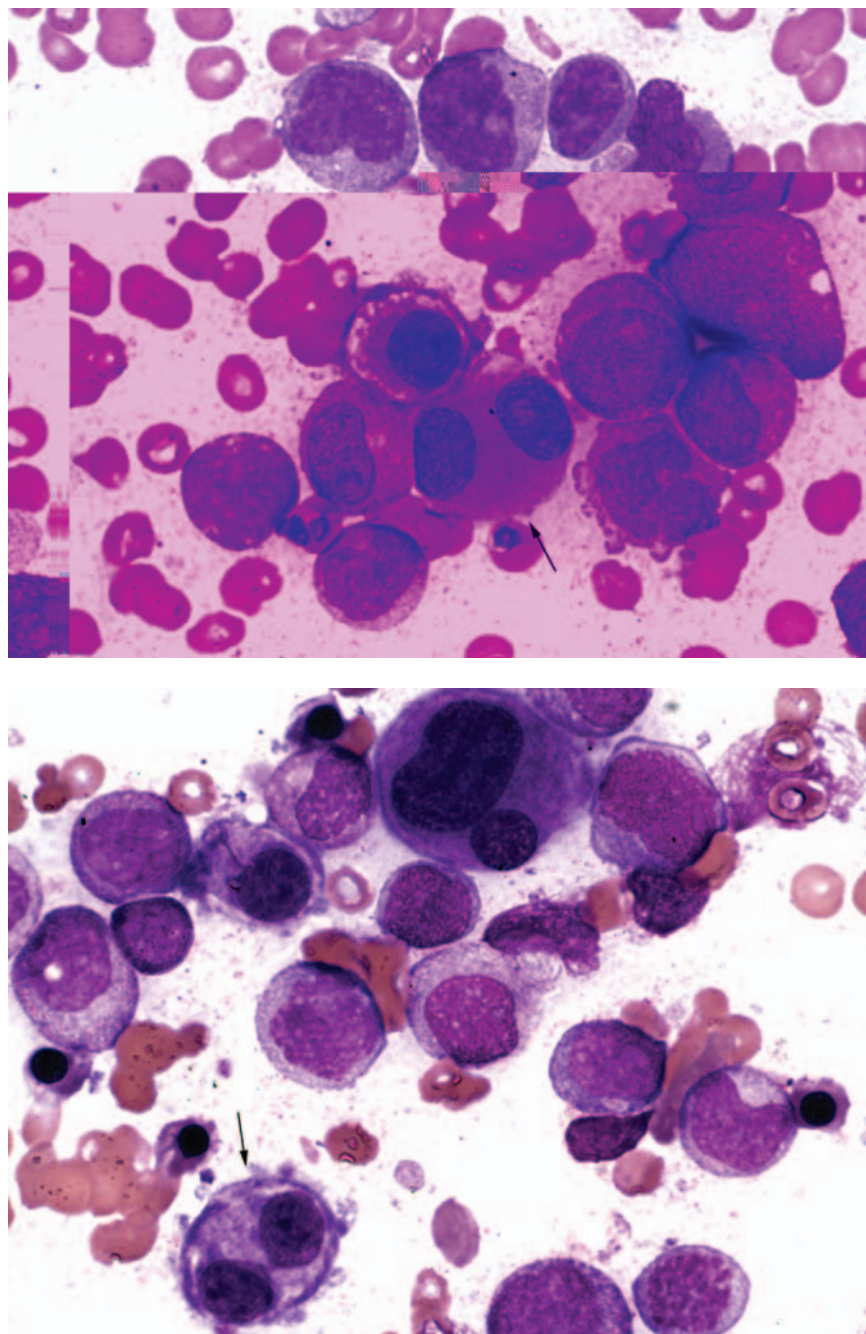
TABLE 11.2 Clinicopathologic features of therapy-related AML.*

Features	Alkylating agents	Topoisomerase II inhibitors
Latency period	4–5 years	<3 years
Preceded MDS	Often present	Often absent
AML subtype	Variable	Mostly monocytic; sometimes promyelocytic or other types
Cytogenetics	Deletions: Often del(5) and del(7)	Translocations: t(9;11); t(6;11); t(15;17); t(8;21); t(3;21); t(6;9)
Median survival time	<8 months	>8 months

* Adapted from Ref. [4].

reason for chemotherapy or irradiation is usually a primary malignancy. In a report by the University of Chicago of 306 patients with t-MDS and t-AML, 25% had Hodgkin lymphoma, 23% had non-Hodgkin lymphoma, and 38% had solid tumors as the primary malignancies [132]. Breast cancer was the most common solid tumor accounting for 10% of the total cases. Approximately 6% of the patients had no prior malignancy and underwent cytotoxic chemotherapy for autoimmune disorders.

The latency period between the initiation of chemotherapy and/or the irradiation and development of t-MDS or t-AML ranges from several months to several years. Overall, the latency period is shorter in patients treated with

**FIGURE 11.22** Acute myeloid leukemia with multilineage dysplasia. Bone marrow smears demonstrating dysplastic immature myelomonocytic cells and binucleated micromegakaryocytes (arrows).

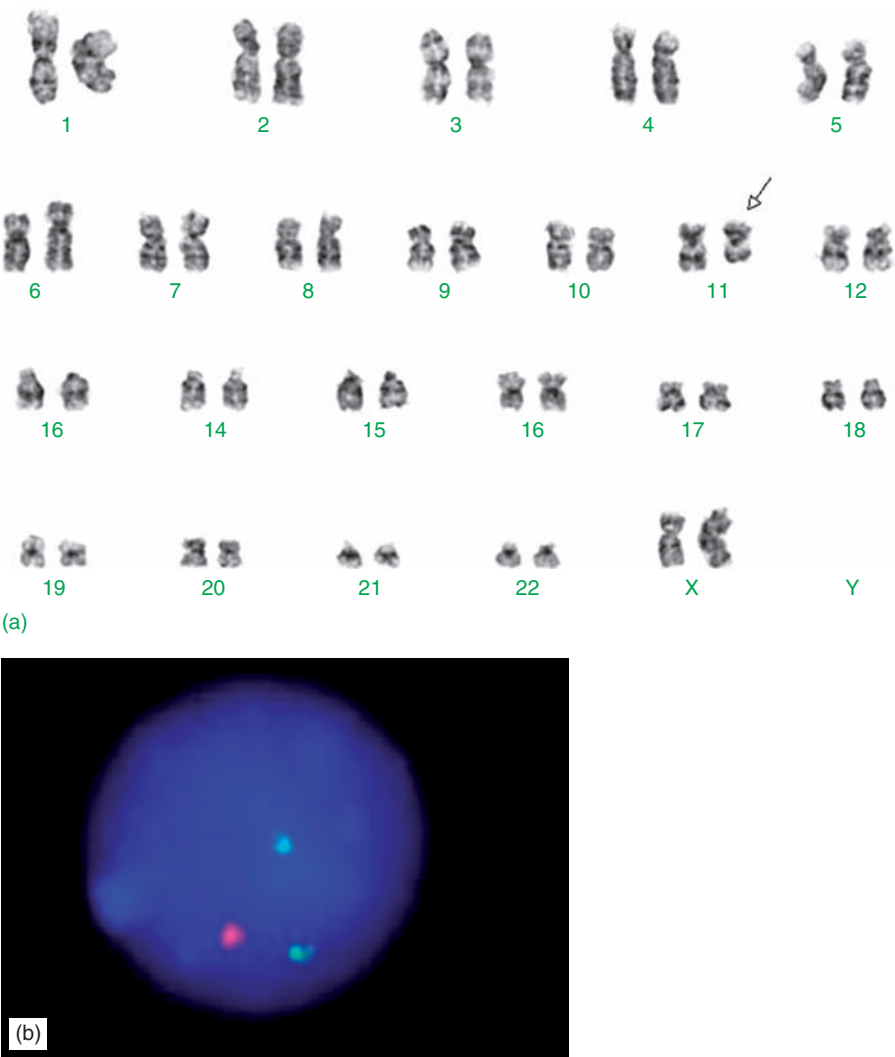


FIGURE 11.23 G-banded karyotype (a, arrow) and FISH analysis (b) showing del(11q) in a patient with acute myeloid leukemia with multilineage dysplasia.

topoisomerase II inhibitors than those treated with alkylating agents or radiation, and longer in younger patients and patients with a non-malignant primary diagnosis [1, 132, 133]. The presence of more than one prior malignancy before diagnosis of t-MDS/t-AML in patients raises the possibility of a constitutional defect predisposing to t-MDS/t-AML.

The primary feature separating t-MDS from t-AML is the percentage of blast counts which is <20% in t-MDS and ≥20% in t-AML. Some of the patients, particularly those treated with topoisomerase II inhibitors, may bypass the MDS phase (Table 11.3).

Alkylating Agent/Radiation-Related AML

Alkylating agent/radiation-related AML has a latency period of about 5–6 years and is usually (>70%) preceded by MDS [1, 133, 135]. The average time for progression from MDS to AML is about 5 months [132]. The occurrence rate appears to be dependent on the age of the patient and the total accumulative dose of the chemotherapeutic agents and/or radiation [1].

TABLE 11.3 Features distinguishing acute promyelocytic leukemia from acute monocytic leukemia.

Features	AML-M3	AML-M5
Azuropophilic granules	More frequent	Less frequent
Auer rods	Frequent	Rare
MPO stain	Strong	Weak
NSE	Negative/weak	Strong
CD4	Negative	Often positive
CD14	Negative	Positive
HLA-DR	Negative	Positive

Pathology

Morphology

The characteristic morphologic features are dysplastic hematopoiesis and increased blasts [1, 136]. Dysplastic changes are usually multilineage and involve myeloid, erythroid, and megakaryocytic series. Hypogranulation and

abnormal segmentation of the granulocytic cells, megaloblastic changes in the erythroid series with ringed sideroblasts, and the presence of micromegakaryocytes are frequent findings. Bone marrow basophilia is sometimes present. Blasts (including promonocytes) are increased ($\geq 20\%$) and often depict dysplastic changes. Morphologically, most t-AML cases correspond to AML with maturation, but a minority of the cases fit into acute myelomonocytic, acute monocytic, acute erythroleukemia, or acute megakaryocytic leukemia (discussed later).

Bone marrow is often hypercellular, but in about 25% of the cases is hypocellular. Bone marrow fibrosis may be present in one-fourth of the cases.

The peripheral blood may show anemia or pancytopenia with aniso-poikilocytosis and leukoerythroblastic features and presence of blast cells.

Immunophenotype

Flow cytometry shows a population of blast cells expressing myeloid-associated markers. CD34 and CD117 are frequently expressed. In some cases, blasts may show aberrant expression of CD4, CD7, and/or CD56. The hypogranularity of the myeloid cells may lead to a lower SSC in the flow cytometric dot plot preparations. Similar to the MDS cases, there may be increased expression of CD11a and CD66 and reduced expression of CD10 and CD116 in the granulocytic cells.

Immunohistochemistry may help to estimate the blast component and the myeloid proportion of the bone marrow cells by using CD34, CD117, and MPO stains.

Molecular and Cytogenetic Studies

Over 90% of the alkylating agent-/radiation-related AMLs show clonal chromosomal aberrations, most frequently involving loss of all or part of chromosome 5, chromosome 7, or both (see Chapter 8). Balanced chromosomal translocations are rare and mostly involve 11q23 or 21q22 (Table 11.2). Some reports show an association between radiation t-AML and t(15;17) or inv(16) [137].

Clinical Aspects

The latency period between the diagnosis of the primary disease and the occurrence of t-AML appears to be longer in the younger patients and patients who have been treated by alkylating agents for non-malignant conditions, such as autoimmune disorders. In the report of the University of Chicago series [132], the median latency period was 82 months and 130 months for the patients ≤ 50 years of age and patients with a non-malignant primary diagnosis, respectively. The overall median latency period for the entire t-AML patient population was 65 months with a median survival of 6.9 months. Patients with chromosomal deletion of 5 and/or 7 had a shorter median survival time than those with chromosomal translocations.

Topoisomerase II Inhibitor–Related AML

Topoisomerase II inhibitor–related AML generally has a shorter latency period than the alkylating agents–related

AML, ranging from 1 to 3 years [1, 132, 133, 135]. Anthracyclines, doxorubicin, etoposide, epipodophyllotoxins, and teniposide are among the major drugs targeting DNA-topoisomerase II.

Pathology

Morphology

The morphologic features most commonly represent acute myelomonocytic or acute monocytic leukemias (discussed later). But some cases may present morphologic and cytogenetic findings consistent with APL [134, 137]. An antecedent myelodysplastic phase is usually lacking.

Immunophenotype

See immunophenotypic features of APL, acute myelomonocytic leukemia, and acute monocytic leukemia in this chapter.

Molecular and Cytogenetic Studies

Topoisomerase II inhibitor–related AML is commonly associated with chromosomal translocations, particularly involving 11q23 and the *MLL* gene [138, 139]. The 11q23-associated cytogenetic changes include del(11q23), (6;11)(q27;q23), t(9;11)(p22;q23), (10;11)(p12;q23), and t(11;19)(q23;p13.1) [138]. Other cytogenetic abnormalities such as t(15;17)(q11;q12), (3;21)(q26;q22), t(8;21)(q22;q22), t(6;9)(p23;q34), and t(8;16)(p11.2;p13) have also been reported in topoisomerase II inhibitor–related AMLs [140–145].

Clinical Aspects

The overall latency period is shorter and the median survival time is longer for the topoisomerase II inhibitor–related AML than for the alkylating agent–related AML.

AMLs NOT OTHERWISE CATEGORIZED

This category represents acute leukemias that are excluded from all previously described subclasses. The primary distinguishing features of leukemias in this category include lineage differentiation and the extent of maturation based on morphological, immunophenotypic, and cytochemical characteristics. Therefore, it includes most of the AML subtypes defined by the French–American–British (FAB) classification including M0, M1, M2, M4, M5, M6, and M7 variants plus acute basophilic leukemia (ABL) and acute panmyelosis with myelofibrosis (APMF).

The criteria for diagnosis of acute leukemia in this category are similar to the previously described subclasses and is based on the WHO requirement of the presence of 20% or more blast cells in the bone marrow or blood differential counts. In addition to myeloblasts, “blast” counts in certain categories of AML may include monoblasts, megakaryoblasts, promonocytes, or promyelocytes. WHO recommends to count 500 nucleated cells on the bone

marrow smears and/or 200 on the blood smears in order to establish a diagnosis of AML.

AML, Minimally Differentiated

Minimally differentiated AML (AML-M0) is defined as an AML with no morphologic or cytochemical evidence of myeloid differentiation based on conventional light microscopic examinations [1]. The myeloid lineage in this category is established by immunophenotypic characteristics and/or ultrastructural studies.

Etiology and Pathogenesis

The etiology and pathogenesis of minimally differentiated AML are not known. No recurrent cytogenetic or molecular abnormalities have been reported in this leukemia, although >50% of the cases may show a variety of chromosomal aberrations (discussed later).

Pathology

Morphology

The leukemic blasts lack features of morphologic differentiation [1–4]. They are often medium sized with scant non-granular basophilic cytoplasm, round or slightly irregular nuclei, fine chromatin, and one or more prominent nucleoli (Figure 11.24). Type II and III myeloblasts are absent or extremely rare (<3%), and Auer rods are not present. Special cytochemical stains, such as MPO, Sudan Black B, and NSE, are negative ($\leq 3\%$ blasts show positive staining). Bone marrow is usually hypercellular and packed with leukemic blast cells, but remnants of normal hematopoietic cells may be noted. The presence of residual normal-maturing myeloid precursors may create a morphologic pattern mimicking AML with maturation (AML-M2). The distinguishing features between AML with minimal differentiation and AML with maturation are lack of type I and II myeloblasts, absence of Auer rods, and negative cytochemical staining in the former (discussed later).

Immunophenotype

Flow cytometric studies reveal expression of myeloid-associated markers, such as CD13, CD33, and/or CD117. CD34 and HLA-DR are often positive, whereas CD38 is negative in a significant proportion of the cases. The CD34⁺/CD38[–] phenotype reflects the very early stage of differentiation in the leukemic blast cells. Most reports show cytoplasmic MPO expression by flow cytometry in leukemia cells in the majority of the cases [146–149]. Monocytic-associated markers, such as CD11c and CD14, are usually negative. Approximately 50% of the cases may express TdT and/or CD7 and about 20% are positive for CD56 [150–152]. The blast cells in occasional cases may express CD2, CD10, or CD19, but cytoplasmic CD3, CD22, and CD79a are negative.

Immunohistochemical stains are negative for CD68, CD3, and CD20, but may show positive reaction for MPO in the blast population.

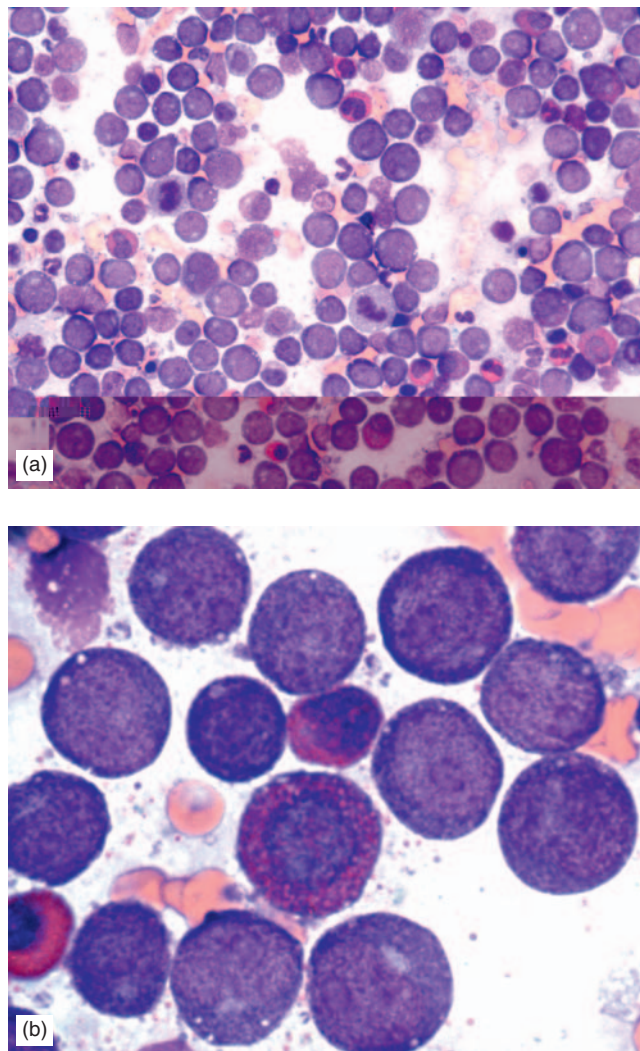


FIGURE 11.24 Acute myeloid leukemia, minimally differentiated. Bone marrow smears (a, low power; b, high power) showing numerous blasts with round nuclei, fine nuclear chromatin, and dark-blue cytoplasm. No cytoplasmic granules or Auer rods are present. These blasts were negative for MPO, Sudan Black B, and NSE, but expressed CD33 and CD117 by flow cytometry.

Molecular and Cytogenetic Studies

A high frequency of mutation in the *RUNX1* (*AML1*) gene has been reported in AML-M0 patients [153–156]. Occasional cases may show BCR/ABL transcript, but *RUNX1*/*RUNXT1* or *CBF β* /*MYH11* transcripts are not found. There is no evidence of T-cell receptor (TCR) or immunoglobulin gene rearrangements.

Cytogenetic aberrations are variable and have been reported in ~50% of the cases. Trisomy 4, 8, 11, 13, and 14 and monosomy 7 are among the most common reported abnormalities [157]. Cases with (9;22)(q34;q11.2), t(11;12)(q23;q24), inv(3)(q21q26), and del(20)q11 (Figure 11.25) are reported less frequently [158–163].

Clinical Aspects

This category of leukemia accounts for <5% of all AMLs in most reported studies. The affected patients are usually older

than 60 years, and the male:female ratio is about 2 [164]. The prognosis is poor with a median survival of <6 months [165]. In one report, the coexpression of CD7 and CD56 was associated with poorer prognosis in patients younger than 46 years [150].

Differential Diagnosis

ALL, AML without maturation, acute monoblastic leukemia, acute megakaryoblastic leukemia (AMKL), and occasionally large cell lymphoma are amongst the list of differential diagnosis. The blast cells in minimally differentiated AML express myeloid-associated markers (such as CD13, CD33, CD117) and show <3% positivity for MPO and Sudan Black B in routine cytochemical stains.

AML without Maturation

Acute myeloid leukemia without maturation (AML-M1) is defined as an acute leukemia with no significant myeloid maturation and $\geq 90\%$ blast cells in the non-erythroid population [1]. The myeloid nature of blast cells is confirmed by positive ($\geq 3\%$) staining for MPO and/or Sudan Black B by cytochemical techniques as well as expression of myeloid-associated markers by immunophenotypic studies.

Etiology and Pathogenesis

The etiology and pathogenesis of AML without maturation are not known. No recurrent cytogenetic or molecular abnormalities have been observed for this leukemia, although trisomy 13 has been reported in some cases [166].

Pathology

Morphology

The morphologic features of the blast cells overlap with those described in AML with minimal differentiation except for (1) the presence of blast cells with some azurophilic cytoplasmic granules and (2) positive MPO and/or Sudan Black B cytochemical staining in $\geq 3\%$ of the blast cells (Figure 11.26) [1–4]. Auer rods are not present.

Similar to most other AMLs, bone marrow is hypercellular and packed with blasts. Variable degrees of marrow fibrosis may be present in a minority of the cases.

Immunophenotype

Flow cytometric studies reveal the expression of myeloid-associated markers, such as CD13, CD33, and/or CD117. CD34 and HLA-DR and cytoplasmic MPO are often positive. Monocytic-associated markers, such as CD11c and CD14, are usually negative. Also, cytoplasmic CD3, CD22, and CD79a are negative.

Immunohistochemical stains are negative for CD68, CD3, and CD20 but may show positive reaction for MPO in the blast population.

Molecular and Cytogenetic Studies

There are reports suggesting a reciprocal exchange between *D12S158* at 12p13.3 and the *MYH11* gene at 16p13 in AML-M1 leukemia [167].

Cytogenetic aberrations are variable and include both numerical (aneuploidy) abnormalities and translocations. Trisomy 11, trisomy 13, and trisomy 14 as well as t(9;12) (q34;p13), t(11;19) (q23;p13), t(14;17) (q32; q11.2), der(12) t(12;17)(p13;q11.2), and der(16)t(16;20)(p13;p11.2) have been reported in this leukemic subtype [157, 166, 168, 169].

Clinical Aspects

AML without maturation accounts for 10–15% of all AMLs and is rare in children. The prognosis is poor, particularly in those with marked leukocytosis and increased circulating blasts.

Differential Diagnosis

The major differential diagnosis of AML without maturation includes ALL, minimally differentiated AML, acute monoblastic leukemia, and AMKL. The blast cells in AML without maturation express myeloid-associated markers (such as CD13, CD33, and CD117) and show $\geq 3\%$ positivity for MPO and Sudan Black B cytochemical stains.

AML with Maturation

Acute myeloid leukemia with maturation (AML-M2) is defined as an acute leukemia with $\geq 20\%$ blast cells in the bone marrow and/or peripheral blood and evidence of granulocytic maturation [1]. The maturing non-blast granulocytic cells account for $\geq 10\%$ and monocytic cells are $\leq 20\%$ of the bone marrow cells. The myeloid nature of blast cells is confirmed by positive ($\geq 3\%$) staining for MPO and/or Sudan Black B by cytochemical technique, as well as expression of myeloid-associated markers by immunophenotypic studies.

Etiology and Pathogenesis

The etiology and pathogenesis of AML with maturation are not known. About 40% of the AML-M2 type shows association with t(8;21)(q22;q22) involving fusion of the *RUNX1* (*AML1*) and *RUNX1T1* (*ETO*) genes. As mentioned earlier, loss of the *AML1* or *CBF β* gene in mice leads to defective hematopoiesis and embryonic death. Also, *RUNX1*/*RUNX1T1* fusion product reduces apoptosis by activating the expression of the anti-apoptosis gene *BCL-2* [170, 171].

Pathology

Morphology

The myeloblasts are large, often with indented nuclei and basophilic cytoplasm. Type II and III myeloblasts are prominent and some blasts may contain large granules mimicking cytoplasmic granules seen in the Chediak-Higashi syndrome [1–4, 172]. Auer rods are frequent and may also be detected in the more mature myeloid forms. Promyelocytes, myelocytes, metamyelocytes, bands, and segmented neutrophils are present and often show dysplastic changes (Figure 11.7). Eosinophilia is common, and some cases may show increased bone marrow basophils and/or mast cells.

The bone marrow biopsy sections are often hypercellular and packed with blasts. Occasionally, bone marrow

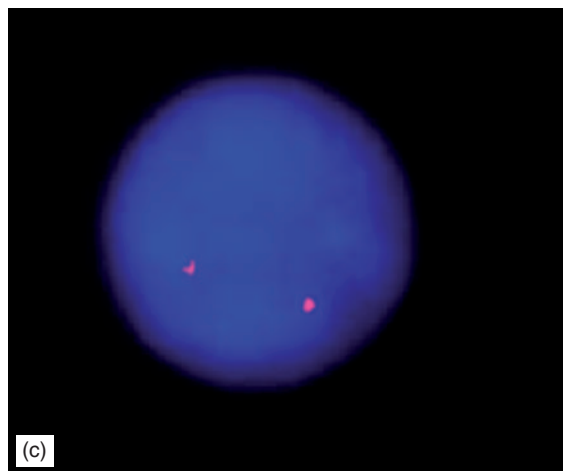
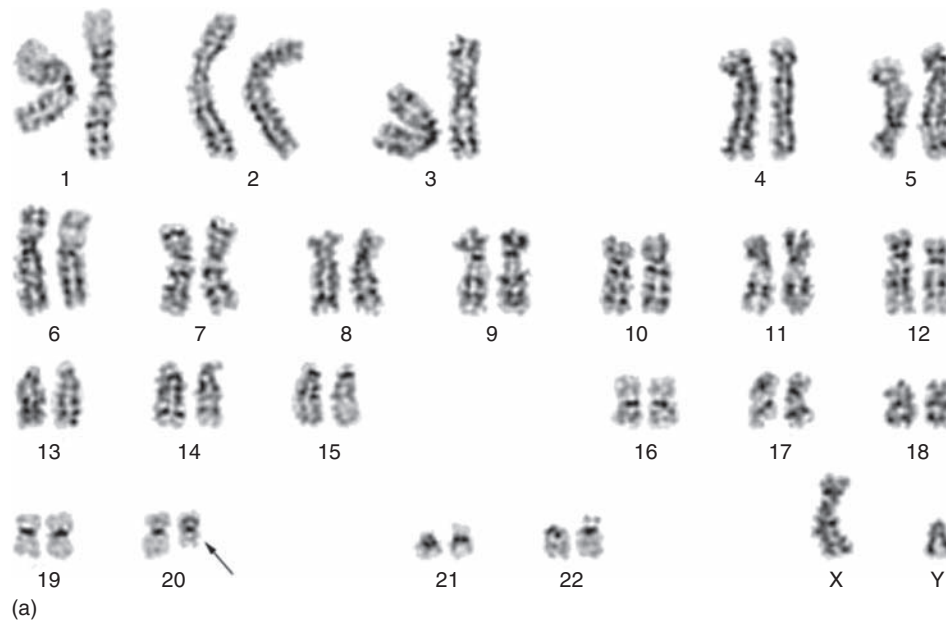


FIGURE 11.25 G-banded karyotype (a, arrow) and FISH analysis (b) showing del(20q) in a patient with AML-M0. (c) A normal cell with two red (20q-specific) signals .

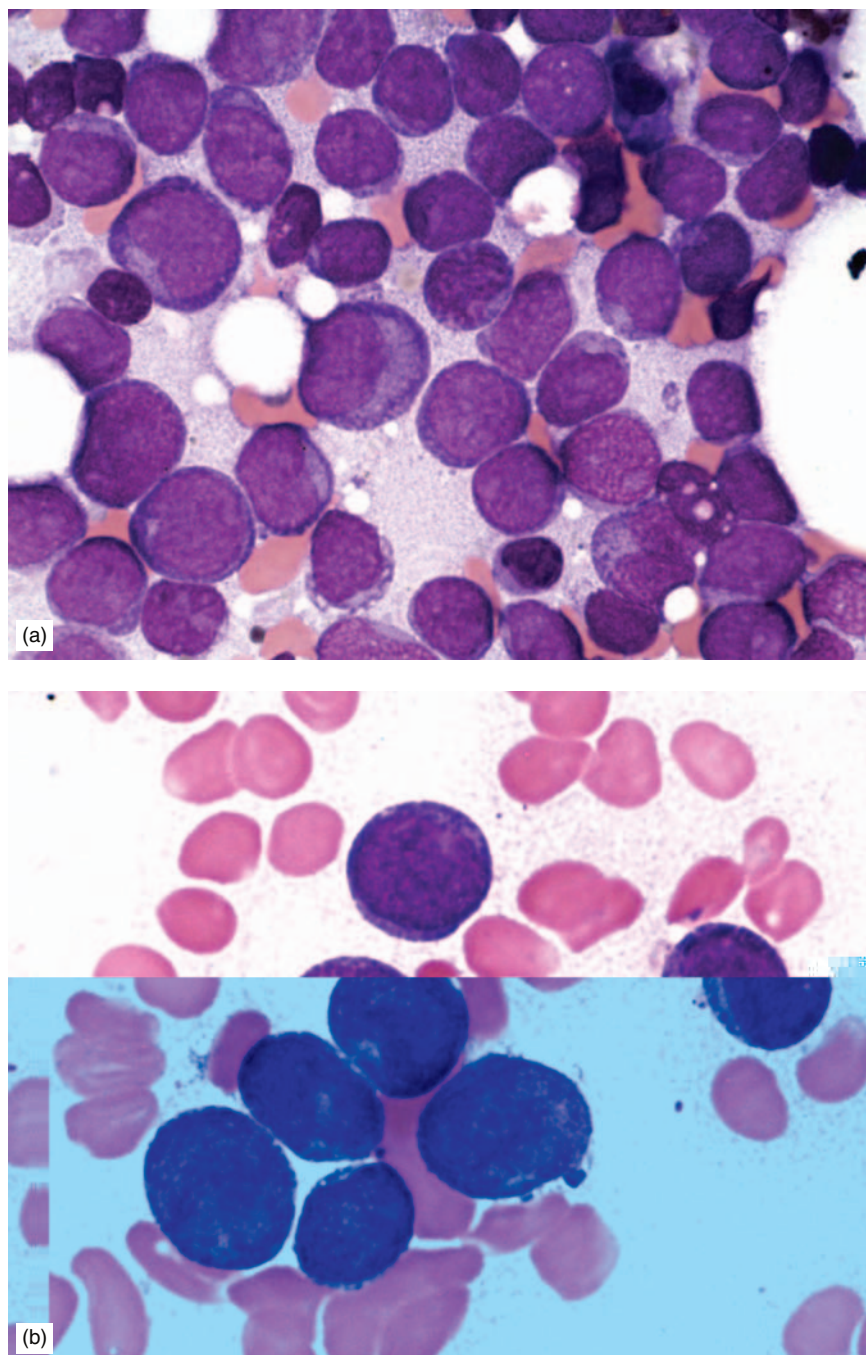


FIGURE 11.26 Acute myeloid leukemia without maturation. Bone marrow smears (a, low power; b, high power) showing numerous blasts with round nuclei, fine nuclear chromatin, and dark blue cytoplasm. There is no evidence of maturation. Auer rods are not present. More than 3% of the blast cells were positive for MPO and Sudan Black B.

may appear normocellular or hypocellular. Variable degrees of marrow fibrosis may be present in a minority of cases.

Immunophenotype

Flow cytometric studies reveal the expression of myeloid-associated markers, such as CD13, CD33, and/or CD117. CD34, HLA-DR, and cytoplasmic MPO are often positive. Monocytic-associated markers, such as CD11c and CD14, are usually negative or present in $\leq 20\%$ of the bone marrow cells. The lymphoid-associated markers, such as CD3, CD20, CD22, CD79a, are usually negative, but there may be aberrant expression of CD19 and/or CD56 [173, 174].

Blast cells may show dim expression of TdT in a small proportion of cases.

Immunohistochemical stains are negative for CD3 and CD20 but may show positive reaction for MPO in the blast population. Scattered immature cells ($<20\%$) may express CD68 in occasional cases.

Molecular and Cytogenetic Studies

As mentioned earlier in this chapter, $t(8;21)(q22;q22)$ accounts for $\sim 40\%$ of the chromosomal aberrations in the cases of AML with maturation (Figure 11.8). For this reason, cytogenetic studies for $t(8;21)$ and molecular monitoring by

RT-PCR are frequently used for diagnosis and detection of minimal residual disease in AML-M2 [175]. Other reported cytogenetic abnormalities include t(6;9)(p23;q34), t(2;9)(q14;p12), t(5;11)(q35;q13) (Figure 11.27), t(10;11)(p13;q14), t(8;19)(q22;q13), t(8;16)(p11;p13), del(12)(p11→p13), and various complex translocations [69, 140, 175–181].

Clinical Aspects

This category of leukemia accounts for 30–40% of all AMLs and occurs in both children and adults. It is the most frequent AML in children. The ones with t(8;21) have a more favorable prognosis in adults with an expected disease-free survival of about 2 years. The clinical outcome for children and cases with other types of chromosomal aberrations is poor.

Differential Diagnosis

The major differential diagnosis includes RAEB, APL, and acute myelomonocytic leukemia. Immunophenotypic and cytogenetic studies are helpful to reach to a definitive diagnosis. Blast cells in AML with maturation often express CD13, CD33, and/or CD117 and lack CD14 expression. They often show t(8;21)(q22;q22) by cytogenetic and molecular analyses.

Acute Myelomonocytic Leukemia

Acute myelomonocytic leukemia (AML-M4) is defined as an acute leukemia with increased immature granulocytic and monocytic cells. Myeloblasts, monoblasts, and promonocytes account for $\geq 20\%$ of the total bone marrow nucleated cells and/or peripheral blood differential counts [1].

Etiology and Pathogenesis

The etiology and pathogenesis of acute myelomonocytic leukemia are not known. The CBF β /MYH11 fusion protein, associated with the chromosome 16 aberrations observed in a majority of these patients, appears to induce granulocytic dysplasia in experimental animals [182, 183]. Also, the translocation of the *MLL* gene, associated with 11q23 aberrations, results in a chimeric gene product which may play a role in leukemogenesis [184, 185].

Pathology

Morphology

The bone marrow smears show myeloid left shift and increased number of immature myelomonocytic cells [1–4]. Myeloblasts show scant-to-moderate amounts of dark-blue cytoplasm, some of which containing various numbers of azurophilic granules (types II and III myeloblasts). Auer rods may be present. The nuclei are usually round or oval, but they may be irregular. The nuclear chromatin is fine, and multiple prominent nucleoli are often present.

Monoblasts are usually larger than myeloblasts ($\sim 40\mu\text{m}$) with abundant dark- to light-blue cytoplasm and

scattered fine azurophilic granules. The nucleoli are round, oval, or folded, and the nuclear chromatin is fine. There is often a single large nucleolus, but multiple prominent nucleoli may be present. Promonocytes are larger than monocytes ($\sim 30\text{--}35\mu\text{m}$) and have abundant light blue to gray cytoplasm. Scattered cytoplasmic azurophilic granules and/or cytoplasmic vacuoles may be present (Figure 11.28). The nuclei are delicately folded or convoluted, often with a cerebriform pattern. The nuclear chromatin is fine, and nucleoli are present but not prominent. Bone marrow smears show an increased number ($\geq 20\%$) of promonocytes, monoblasts, and myeloblasts (Figures 11.29 and 11.30).

The bone marrow biopsy sections are often hypercellular and packed with immature myelomonocytic cells (Figure 11.29). Variable degrees of marrow fibrosis may be present in a minority of the cases. Monocytic nodules may be present.

The peripheral blood smears show absolute monocytosis (often $\geq 5,000/\mu\text{L}$) with the presence of promonocytes, left-shifted granulocytic series, and various numbers of circulating blasts (Figure 11.29).

In a significant proportion of patients (5–35%), there is evidence of extramedullary leukemic infiltration, such as involvement of skin, mucosal membranes, lymph nodes, liver, and/or spleen [1, 186].

Immunophenotype and Cytochemical Stains

The immature myelomonocytic population expresses CD13 and CD33 with partial expression of CD11c, CD14, CD15, CD34, CD36, CD64, CD117, HLA-DR, and MPO by flow cytometry (Figure 11.31) [187]. Immunohistochemical stains, such as MPO, lysozyme, and CD68, are used for the evaluation of the bone marrow myelomonocytic component, and CD34 and CD117 stains are often helpful for the estimation of blast cells. Monocytic precursors strongly express CD68 and lysozyme and are negative or weakly positive for MPO. Granulocytic precursors strongly express MPO but are negative for CD68 and show weak or moderately positive reactions for lysozyme. Aberrant expression of CD2, CD7, and/or CD56 has been observed frequently [188–190].

The cytochemical stains show strong MPO positivity for the granulocytic lineage and various degrees of diffuse cytoplasmic NSE staining for the monocytic population.

Molecular and Cytogenetic Studies

In addition to the chromosomal aberrations involving 11q23 and inv(16)(p13q22) [184, 191, 192], a number of non-specific cytogenetic abnormalities have been reported in acute myelomonocytic leukemias. These include dup(1)(p31.2p36.2), t(1;3)(p36;q21), t(8;12)(q13;p13), t(9;21)(q13;q22), and t(6;7)(q23;q35) [192–197].

Clinical Aspects

Acute myelomonocytic leukemia accounts for about 15–25% of all AMLs. The median age is around 50 years, but it may occur at any age. The incidence is slightly more in males than in females. Similar to other acute leukemias, clinical symptoms are the result of bone marrow involvement and extramedullary infiltration by the leukemic cells. Fatigue,

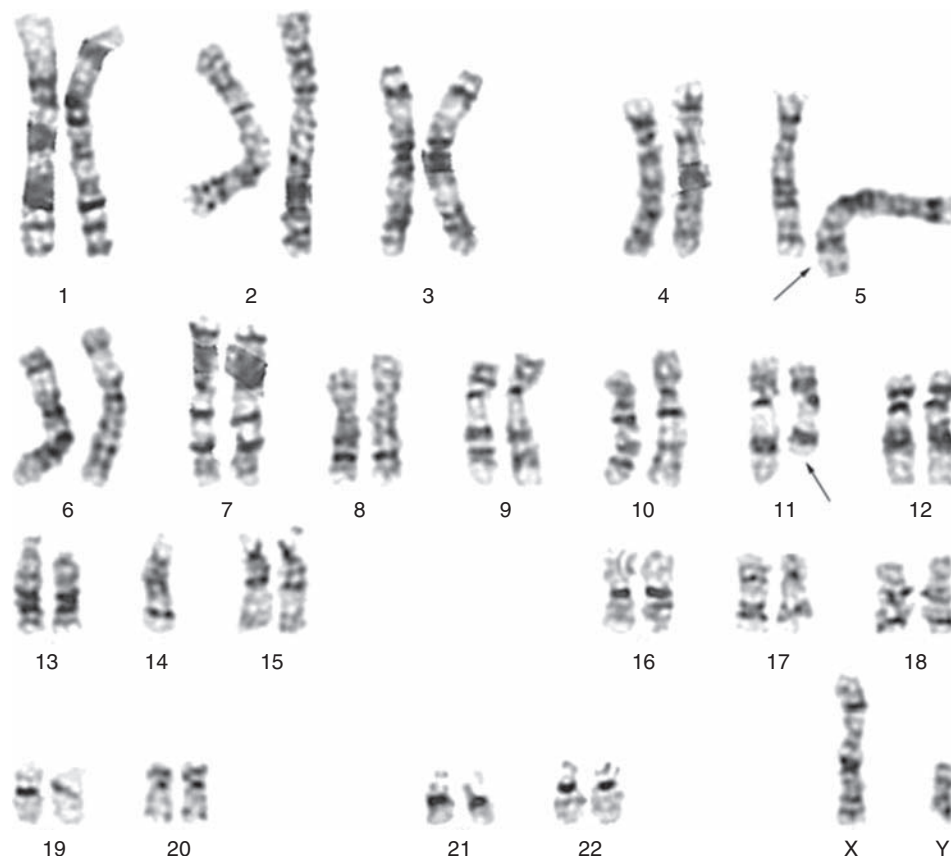


FIGURE 11.27 Translocation of 5;11 in a patient with acute myeloid leukemia with maturation.

fever, bleeding disorders, gingival hyperplasia, lymphadenopathy, hepatosplenomegaly, and skin involvement are among frequent clinical findings. As mentioned earlier in this chapter, patients with *inv(16)* have a favorable prognosis and those with translocation of 11q23 fall into the category of leukemias with intermediate survival rate [104]. Some studies show a correlation between the expression of CD56 by the leukemic cells and severe fatal hyperleukocytosis in patients with acute myelomonocytic leukemia [189]. Successful effect of treatment with NUP98–HOXD11 fusion transcripts and monitoring of minimal residual disease in patients with AML-M4 has been reported [198].

Differential Diagnosis

The differential diagnosis of acute myelomonocytic leukemia includes chronic myelomonocytic leukemia (CMML), AML with maturation, APL, and acute monocytic leukemia. The diagnosis of acute myelomonocytic leukemia is established by the demonstration of the sum of $\geq 20\%$ myeloblasts and monoblastic/promonocytes in the bone marrow or peripheral blood. It is distinguished from the microgranular variant of APL by the expression of NSE, CD4, CD14, and HLA-DR and by the absence of *t(15;17)* (Table 11.3).

Acute Monoblastic and Acute Monocytic Leukemias

Acute monoblastic and acute monocytic leukemias are acute leukemias in which $\geq 80\%$ of the leukemic cells are

of monocytic lineage consisting of monoblasts, promonocytes, and monocytes. When monoblasts are the major cellular component ($\geq 80\%$ of the leukemic cells) the term “acute monoblastic leukemia” (AML-M5a) is used, and when promonocytes and monocytes account for most of the leukemic cells ($\geq 80\%$), the condition is referred to as “acute monocytic leukemia” (AML-M5b) [1].

Etiology and Pathogenesis

The etiology and pathogenesis of acute leukemias of monocytic lineage are not known. As mentioned earlier, translocation of the *MLL* gene, associated with 11q23 aberrations, results in a chimeric gene product which may play a role in the development of acute leukemias of monocytic lineage. Up to 10% of t-AMLs, particularly the topoisomerase II inhibitor–related type, are associated with 11q23 aberrations, suggesting a causative role for chemotherapy/radiation in the development of this disorder.

Pathology

Morphology

Monoblasts usually show abundant dark- to light-blue cytoplasm with no or a few scattered fine azurophilic granules (Figures 11.32 and 11.33). The nucleoli are round, oval, or folded, and the nuclear chromatin is fine. There is often a single large nucleolus, but multiple prominent nucleoli may be present. Promonocytes have abundant light-blue to gray cytoplasm (Figure 11.34). Scattered cytoplasmic

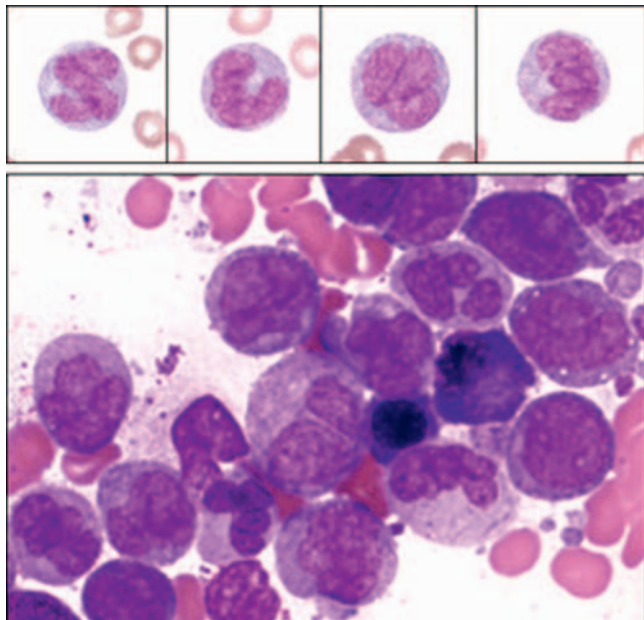


FIGURE 11.28 Promonocytes are counted as blast cells in myeloid leukemias and are prominent in acute myelomonocytic and monocytic leukemias.

azurophilic granules and/or cytoplasmic vacuoles may be present. The nuclei are delicately folded or convoluted, often with a cerebriform pattern. The nuclear chromatin is fine and nucleoli are present but not prominent. Auer rods are rare. Granulocytic precursors account for $\leq 20\%$ of the bone marrow non-erythroid nucleated cells.

The bone marrow biopsy sections are often hypercellular and packed with blasts and immature myelomonocytic cells. Variable degrees of marrow fibrosis may be present in a minority of cases.

The peripheral blood smears show absolute monocytosis (often $\geq 5,000/\mu\text{L}$) with the presence of monoblasts, promonocytes, and monocytes (Figure 11.33b). The proportion of monocytes and promonocytes in blood smears may sometimes be much greater than the blast cells as compared to the bone marrow smears.

Extramedullary leukemic infiltration by leukemia cells is relatively common, such as involvement of gum, skin (Figure 11.35), central nervous system, lymph nodes, liver, and/or spleen [199].

Immunophenotype and Cytochemical Stains

The major difference between acute myelomonocytic leukemia and acute monocytic leukemia is the proportion of monocytic versus granulocytic precursors [1]. Morphologic features alone may not be sufficiently clear to make such a distinction, and in most instances, there is a need for immunophenotyping in order to separate these two entities from each other.

The immature monocytic population expresses CD4, CD11c, CD15, CD14, CD36, CD64, and HLA-DR by flow cytometry [187, 200]. Aberrant expression of CD56 may be present (Figure 11.36). A small proportion of leukemic cells may also express CD13, CD33, CD117, and/or weak MPO. CD34 is usually negative. Immunohistochemical stains are

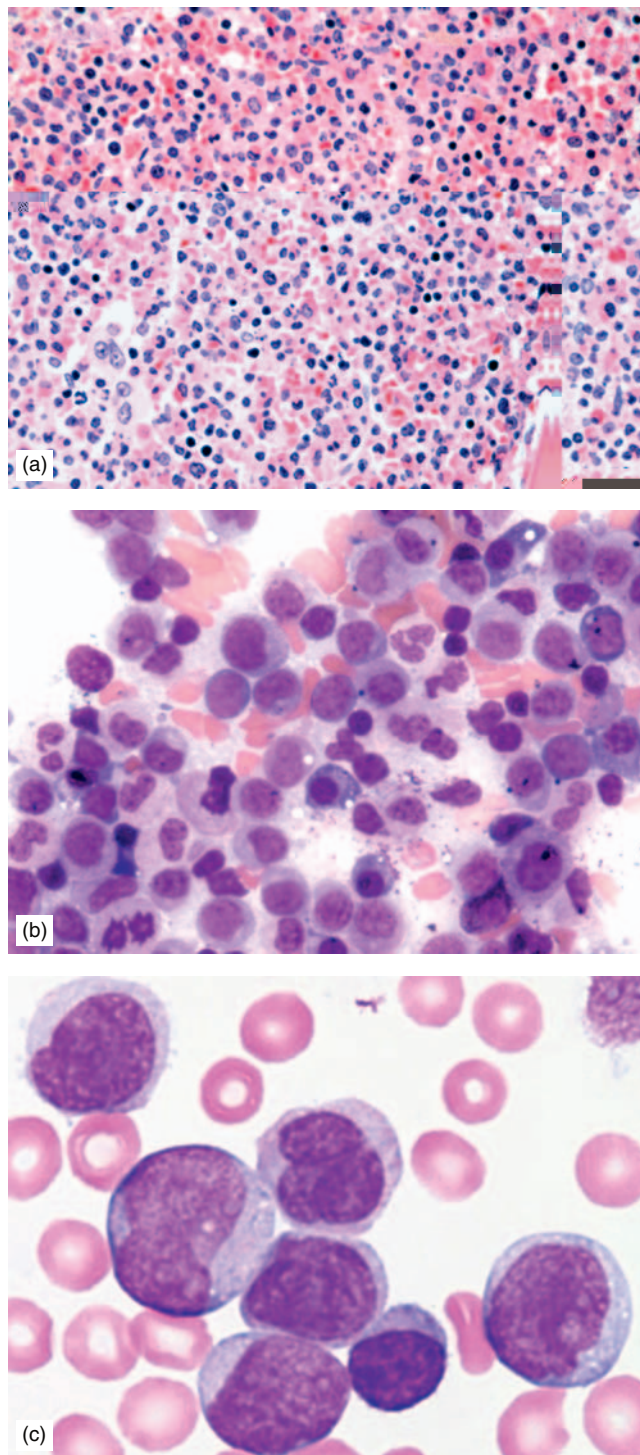


FIGURE 11.29 Acute myelomonocytic leukemia. Biopsy section (a) demonstrating a hypercellular marrow with numerous immature cells with round or irregular nuclei and fine chromatin. Some immature cells show one or more prominent nucleoli. Bone marrow smear depicts myeloid left shift with increased immature myelomonocytic cells and blasts (b). Blood smear shows several blasts and immature monocytic cells (c).

usually positive for lysozyme and CD68 and may show a weak or focal reactivity for MPO.

The cytochemical stains show strong diffuse cytoplasmic NSE staining. Monoblasts are usually MPO-negative, but promonocytes may show a weak positive reaction.

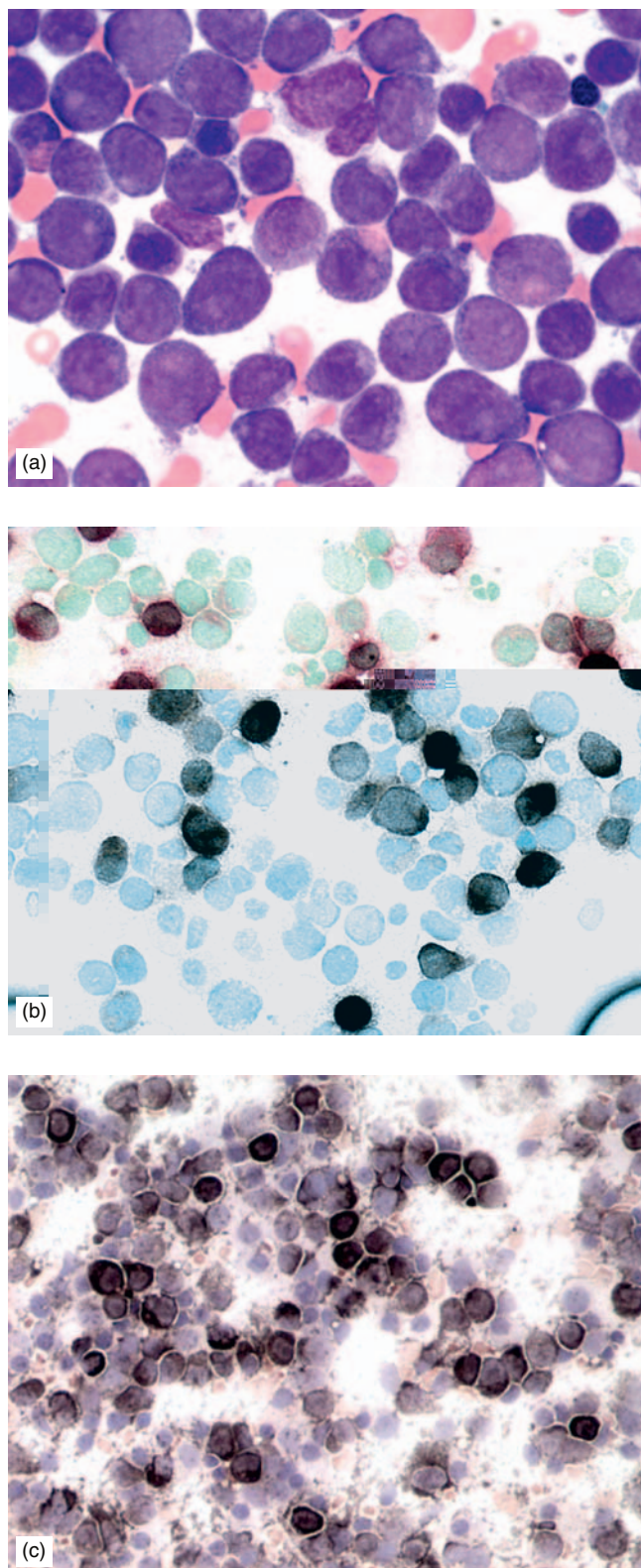


FIGURE 11.30 Acute myelomonocytic leukemia. Bone marrow smear showing increased blasts and immature myeloid cells with round or convoluted nuclei (a). Monoblasts/promonocytes are positive for an NSE stain (b) and myeloblasts are positive for MPO (c).

Molecular and Cytogenetic Studies

Overall, the frequency of chromosomal aberrations, such as translocation of 11q23 and trisomy 8, is significantly higher in monoblastic (M5a) type than the monocytic (M5b) type. In one large study, the incidence of 11q23 (*MLL*) aberrations in M5a and M5b was reported as 33.3% and 15.9%, respectively [185]. Reported cytogenetic abnormalities and affected genes include [83, 201–207]:

$t(11;17)(q23;q21)$; (*MLL-AF17*)

$t(10;11)(p11.2;q23)$; (*ABI-1;MLL*)

$t(11;20)(p15;q11.2)$ (*NUP98-TOP1*)

$ins(X;11)(q24;q23q13)$; (*Septing6-MLL*)

$t(8;16)(q11;p13)$; (*MOZ-CBP*) (Figure 11.37)

$(5;11)(q31;q23q23)$; (*GRAF-MLL*).

Acute monocytic leukemia with $t(8;16)(p11;p13)$ is rare and has been mostly reported in infants and children, often with a bleeding tendency and disseminated intravascular coagulopathy (DIC). The leukemia cells may demonstrate hemophagocytosis and may morphologically mimic the microgranular variant of acute promyelocytic leukemia [204].

Clinical Aspects

Acute leukemias of monocytic lineage account for about 3–6% of all AMLs. The median age is around 50 years, but it may occur at any age. The incidence is higher in men than in women (male:female ratio is about 1.8). Clinical symptoms are the result of bone marrow involvement and extramedullary infiltration by the leukemic cells. Fatigue, fever, bleeding disorders, gingival hyperplasia, lymphadenopathy, CNS involvement, and hepatosplenomegaly are among the frequent symptoms. In one major study, the monocytic type (M5b) had a better clinical outcome than the monoblastic type (M5a) with a reported 3-year disease-free survival of 28% and 18%, respectively [208]. In another study, the complete remission rate and disease-free survival did not differ significantly between patients with M5a and M5b [209].

Differential Diagnosis

The differential diagnosis of acute monoblastic leukemia (AML-M5a) includes ALL, minimally differentiated AML, AML without maturation, AMKL, and large cell lymphoma.

Acute monocytic leukemia (AML-M5b) should be distinguished from chronic myelomonocytic leukemia (CMML), acute myelomonocytic leukemia, and microgranular variant of APL.

Leukemia cells of monocytic/monoblastic origin may express NSE, lysozyme, CD4, CD14, CD64, and/or CD68.

Acute Erythroid Leukemias

Acute erythroid leukemia (AML-M6) is defined as a subtype of AML with predominance of erythroid precursors ($\geq 50\%$ of bone marrow nucleated cells should be of

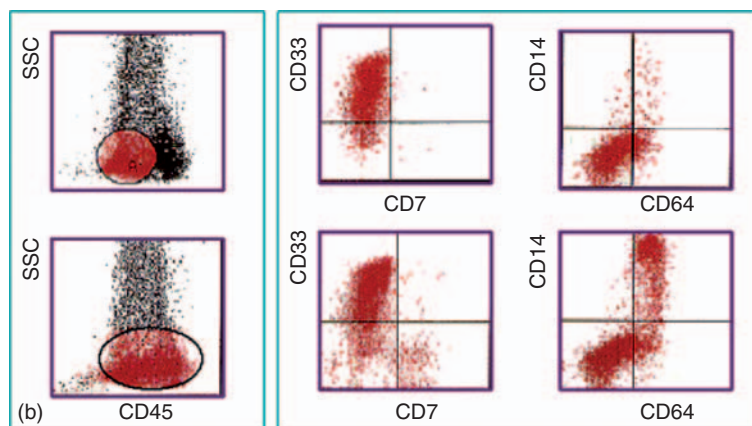
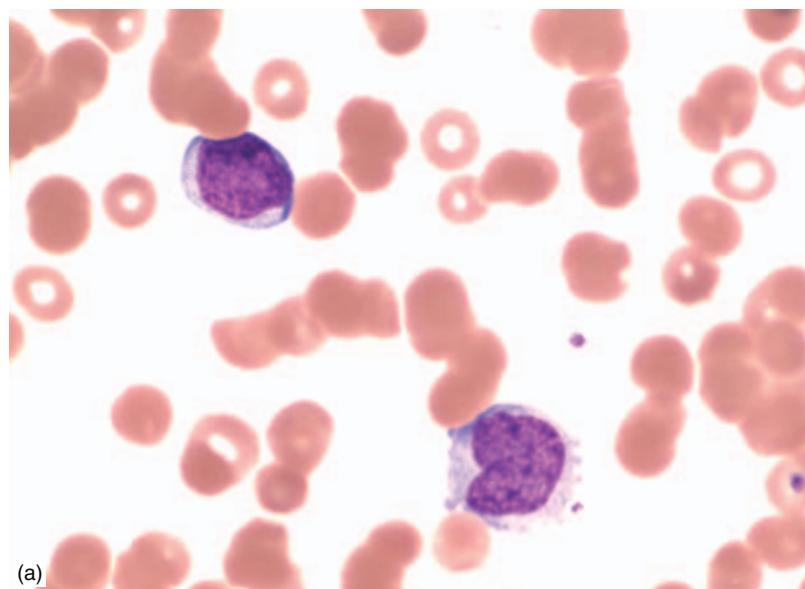


FIGURE 11.31 Acute myelomonocytic leukemia. Peripheral blood smear showing a blast and a promonocyte (a). Flow cytometry demonstrates two gates (b). The upper gate, CD45^{dim} population, expresses CD13 and CD33 and is negative for CD14. The lower gate, CD45^{dim} and CD45^{strong}, demonstrates an additional population of monocytes expressing CD14.

erythroid origin) [1]. Acute erythroid leukemia is divided into two morphologic categories: (1) erythroleukemia, consisting of myeloblasts and erythroid precursors, and (2) pure erythroid leukemia [1, 210, 211].

Etiology and Pathogenesis

The etiology and pathogenesis of erythroid leukemia are not known. Recent studies suggest that the loss of splicing function of the hematopoietic transcription factor Spi-1/PU.1 may play a role in the pathogenesis of erythroid leukemia [212]. A significant number of erythroid leukemias are therapy related or are the result of blast transformation in MDS, and therefore in part share the pathogenesis of t-AML and MDS [213, 214]. It has also been suggested that an inadequate supply of the mutagenic nucleotide of cytosine, possibly through impaired synthesis, could cause both the megaloblastic and the leukemic changes in erythroleukemia [215].

Pathology

Morphology

Multilineage dysplasias, particularly dyserythropoiesis, are common bone marrow features. These include

megaloblastic changes, nuclear budding and fragmentation, multinuclearity, and basophilic stippling in the erythroid precursors. Hypogranulation, abnormal nuclear segmentation, and giant forms may be present in the granulocytic series. Micromegakaryocytes and megakaryocytes with separated nuclei are not infrequent [1, 214, 216–218]. Erythroid leukemia is divided into two subcategories according to the WHO classification.

Erythroleukemia (erythroid/myeloid, AML-M6a): This subtype is defined by at least 50% of the bone marrow nucleated cells being of erythroid origin and $\geq 20\%$ myeloblasts in the non-erythroid component (Figures 11.38 and 11.39) [1, 216, 217]. The erythroid series are dysplastic and left shifted, but are usually found in all stages of maturation. Myeloblasts may be of type I, II, or III or a mixture of all three types. Occasionally, Auer rods are present. Bone marrow iron stores are often increased and ringed sideroblasts may be present. This morphologic category accounts for the majority of acute erythroid leukemias.

Bone marrow biopsy sections are usually hypercellular with clusters or sheets of immature cells and marked reduction in the normal hematopoietic components.

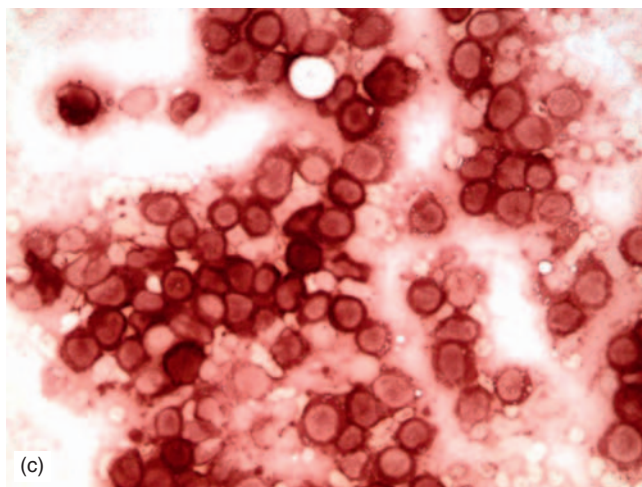
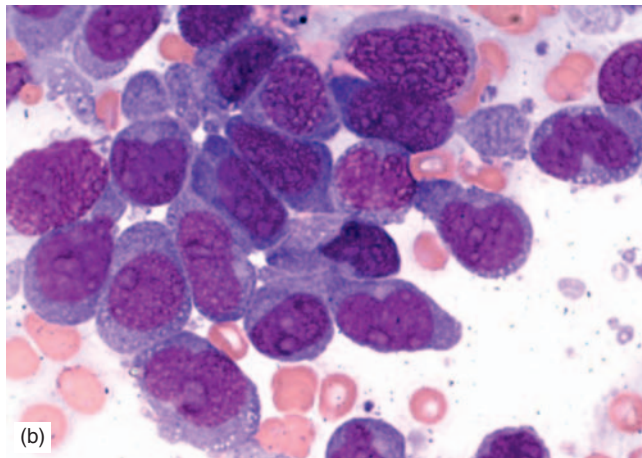
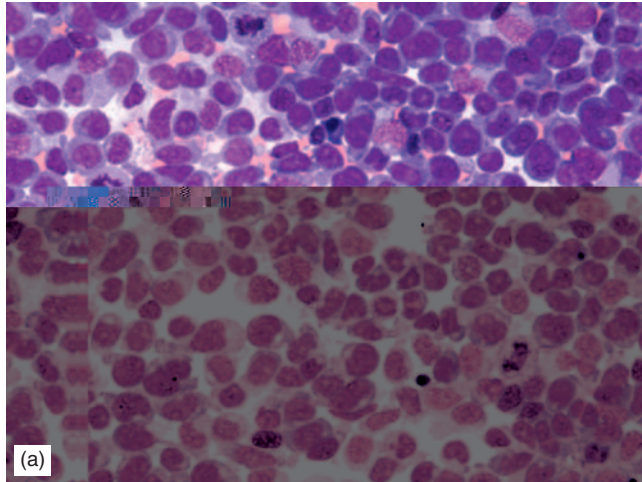


FIGURE 11.32 Acute monoblastic leukemia. Bone marrow smear demonstrates sheets of blast cells with variable amounts of finely vacuolated, blue cytoplasm, round or irregular nuclei, fine chromatin, and one or more prominent nucleoli: (a) low power and (b) high power. The majority of blast cells stain for NSE (c).

Blood smears show aniso-poikilocytosis with the presence of schistocytes, tear-drops, and macrocytes. Basophilic stippling is present. Granulocytic series may show hypogranulation and hyposegmentation. Giant and/or

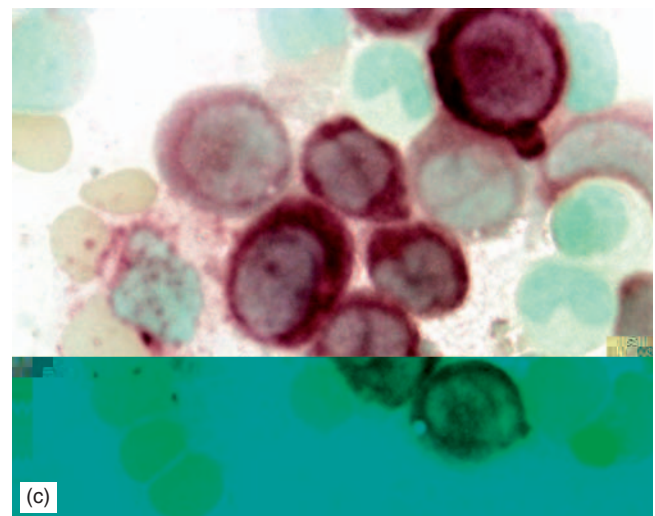
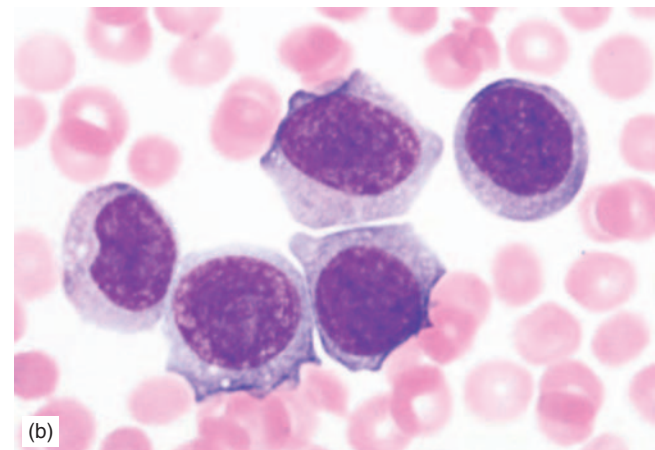
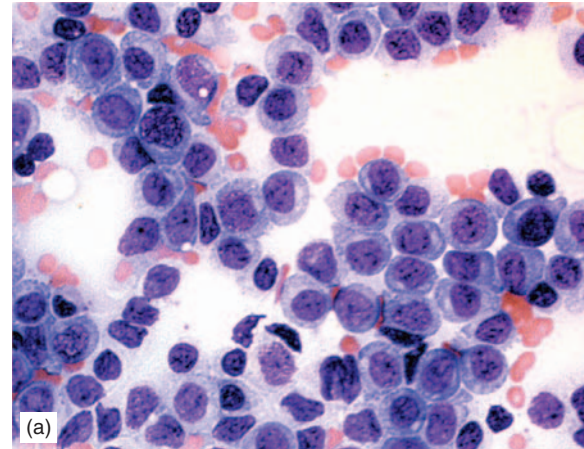


FIGURE 11.33 Acute monoblastic leukemia. Bone marrow smear demonstrates numerous blast cells with abundant blue cytoplasm, round nuclei, fine chromatin, and one or more prominent nucleoli (a). Similar blast cells are present in the peripheral blood smear (b). Blasts are positive with NSE stain (c).

hypogranular platelets are often present. Various numbers of nucleated red blood cells and blasts are often present.

The WHO requirement for the diagnosis of erythroleukemia leaves significant overlapping features with RA

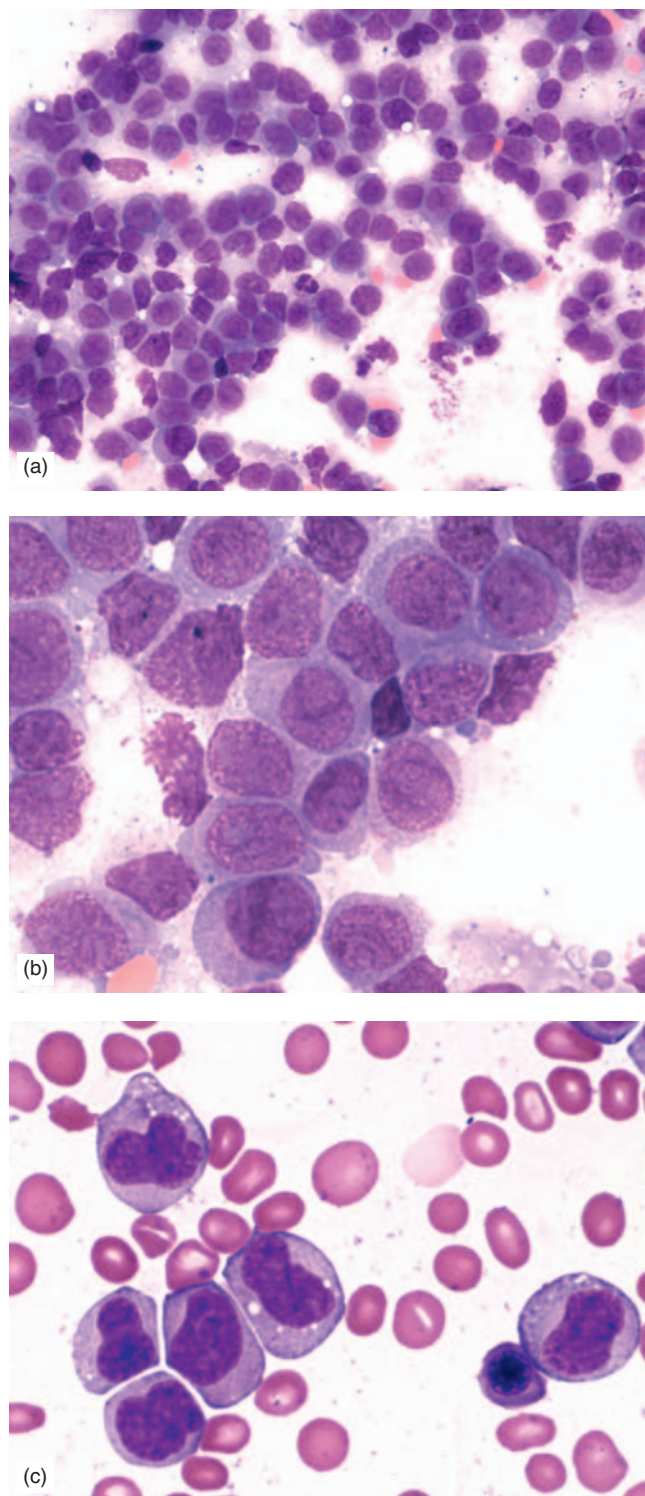


FIGURE 11.34 Acute monocytic leukemia. Bone marrow smear showing increased number of promonocytes with scattered blasts: (a) low power and (b) high power. Peripheral blood smears demonstrate several atypical monocytes (c).

and RAEB. If we follow the WHO requirements, a simple calculation tells us that any bone marrow sample with dysplastic changes, $\geq 50\%$ and $\leq 80\%$ erythroid precursors, and 4% or more myeloblasts in the total bone marrow

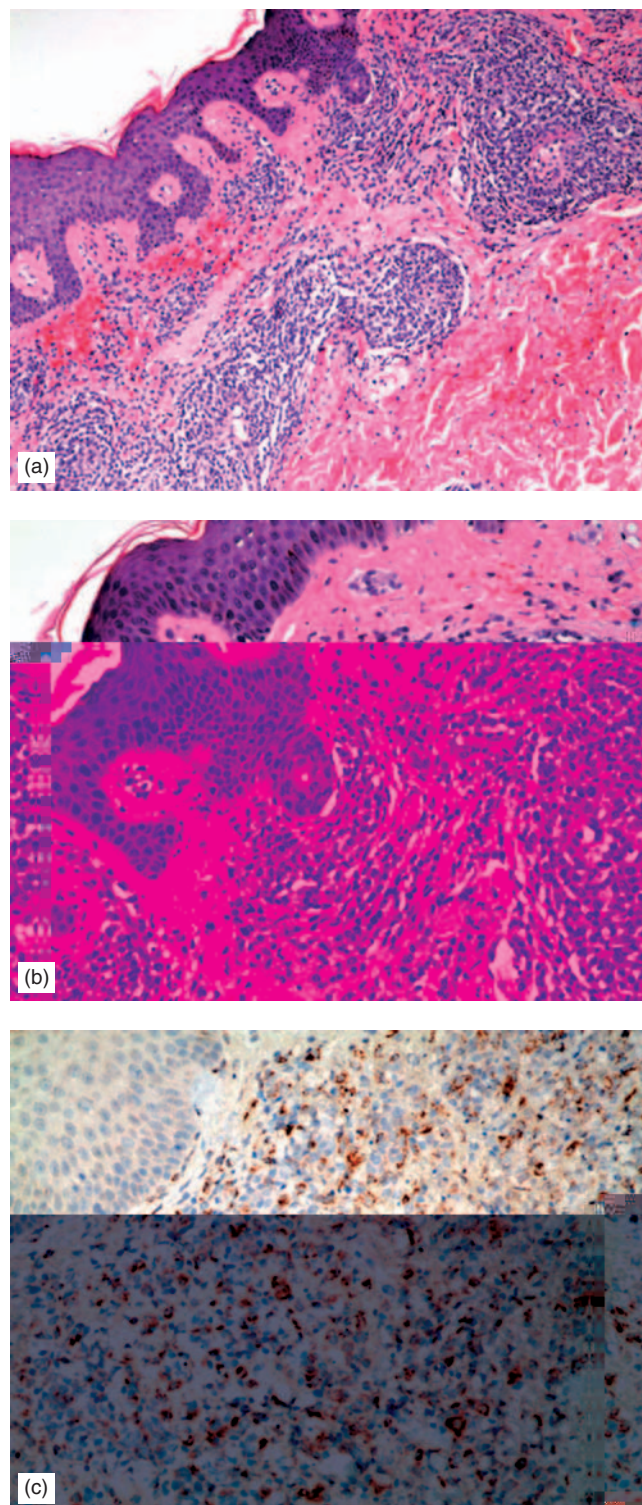


FIGURE 11.35 Dermal infiltration of leukemic cells in a patient with acute monocytic leukemia: (a) low power and (b) high power. Many of the leukemic cells are positive for CD68 by immunohistochemistry (c).

cells is qualified for the diagnosis of acute erythroleukemia. For example, a bone marrow sample with 51% erythroid precursors and 10% myeloblasts in the total bone marrow cells should be diagnosed as erythroleukemia, whereas a bone

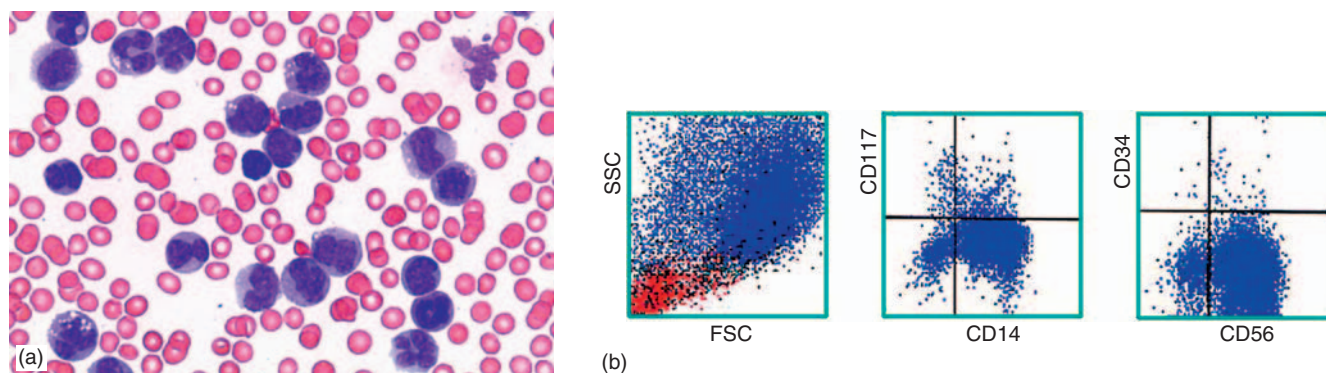


FIGURE 11.36 (a) Peripheral blood smear from a patient with acute monocytic leukemia demonstrating numerous atypical monocytes and promonocytes (bone marrow smears showed $>20\%$ blasts and promonocytes). (b) Flow cytometric studies reveal a large population of monocytic cells which are positive for CD14 and negative for CD34 and CD117. They also aberrantly express CD56.



FIGURE 11.37 G-banded karyotype reveals $t(8;16)$ in a patient with acute monocytic leukemia.

marrow sample with 49% erythroid precursors and 15% myeloblasts is called RAEB II and not an acute leukemia! This problem with the diagnostic criteria for acute erythroleukemia has been raised in the literature. Further clinical investigations are needed for clarification of the status of erythroleukemias with low myeloblast counts [211].

Pure Erythroid Leukemia (AML-M6b): This category is defined as a disorder with $>80\%$ of bone marrow nucleated cells consisting of erythroid precursors (Figures 11.40 and 11.41). These cells are predominantly erythroblasts (pronormoblasts) with dark-blue cytoplasm, round nuclei, fine nuclear chromatin, and one or more prominent nucleoli. The cytoplasm often contains poorly demarcated vacuoles [1, 218]. Evidence of dyserythropoiesis and/or

megaloblastic changes is often present. Pure erythroid leukemia is far less frequent than the erythroleukemia (erythroid/myeloid) type.

Immunophenotype and Cytochemical Stains

The erythroid precursors are often positive for GPA, hemoglobin A, and CD71 (transferring receptor) (Figure 11.42). The very early cells may lack expression of one or more of these markers. HLA-DR and CD34 are often negative. The erythroid precursors do not express myeloid-, monocytic-, or megakaryocytic-associated markers. They are negative for cytochemical MPO, Sudan Black B, and NSE stains, but often show globular or coarsely granular cytoplasmic PAS positivity.

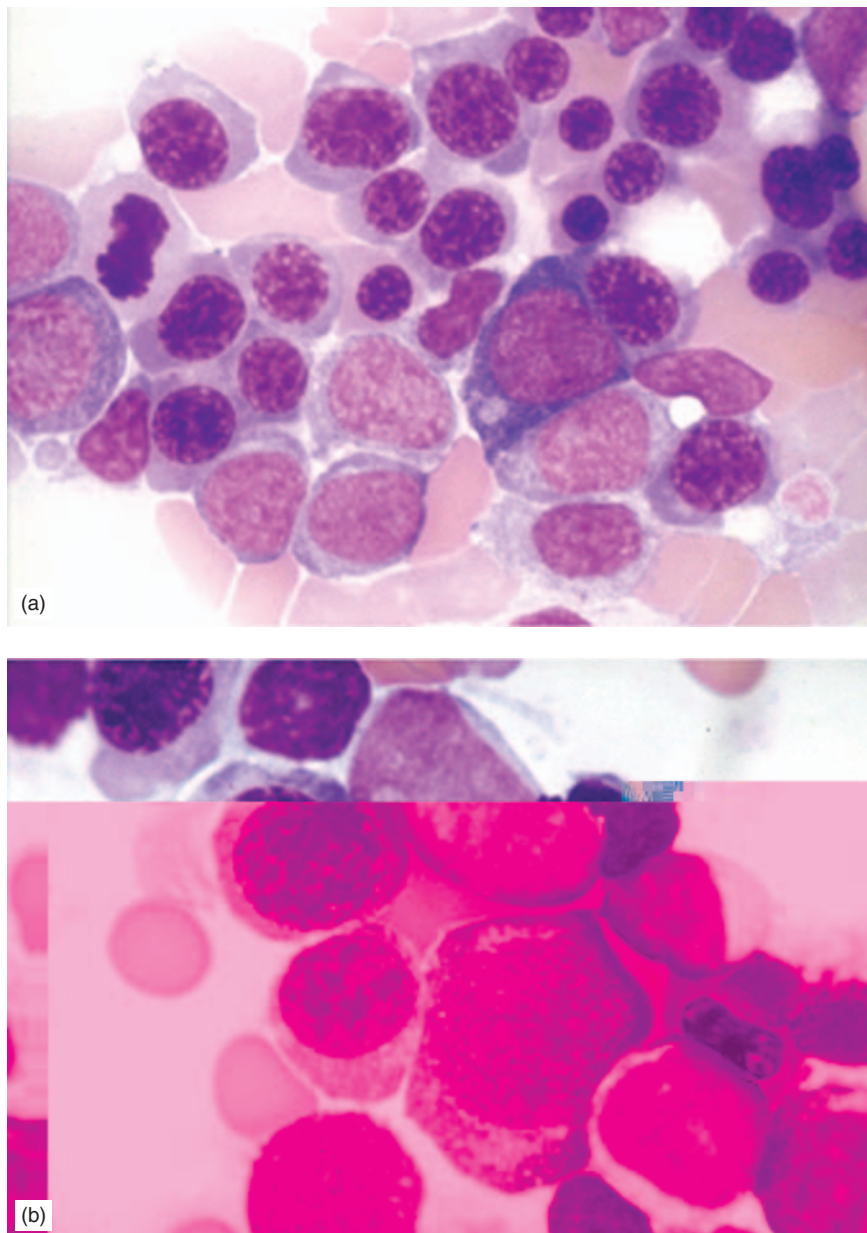


FIGURE 11.38 Erythroid/myeloid leukemia (erythroleukemia (AML-M6a)). Bone marrow smears show erythroid preponderance and left shift with increased myeloblasts: (a) low power and (b) high power.

The myeloblasts in the AML-M6a variant, similar to the myeloblasts in other AMLs, express myeloid-associated markers, such as CD13, CD33, CD117, and MPO, and are often positive for CD34 and HLA-DR.

Molecular and Cytogenetic Studies

Acute erythroid leukemia shares many cytogenetic features with MDS (see Chapter 8). Partial loss or monosomy of chromosomes 5 and 7 is the most frequent chromosomal aberration reported in acute erythroid leukemias, followed by abnormalities of chromosomes 8, 16, and 21 [219–221]. Some of the cases of pure erythroid leukemia are found to be associated with a *BCR-ABL1* fusion. Complex chromosomal abnormalities are frequent findings.

Clinical Aspects

Acute erythroid leukemias account for about 5% of all AMLs. The vast majority (>90%) are of erythroid/myeloid (AML-M6a) subtype. Pure erythroid leukemia is rare. The median age is around 57 years, ranging from 20 to 80 years [222]. The incidence is higher in men than in women (male: female ratio is about 2:1). A significant proportion (up to 50%) of acute erythroid leukemia represents either therapy-related or evolution in patients with a history of MDS [213, 222]. Severe anemia, usually with granulocytopenia and/or thrombocytopenia, is a common feature.

Acute erythroid leukemia is an aggressive disease, but the erythroid/myeloid type (AML-M6a) does significantly better than the pure erythroid leukemia type (AML-M6b).

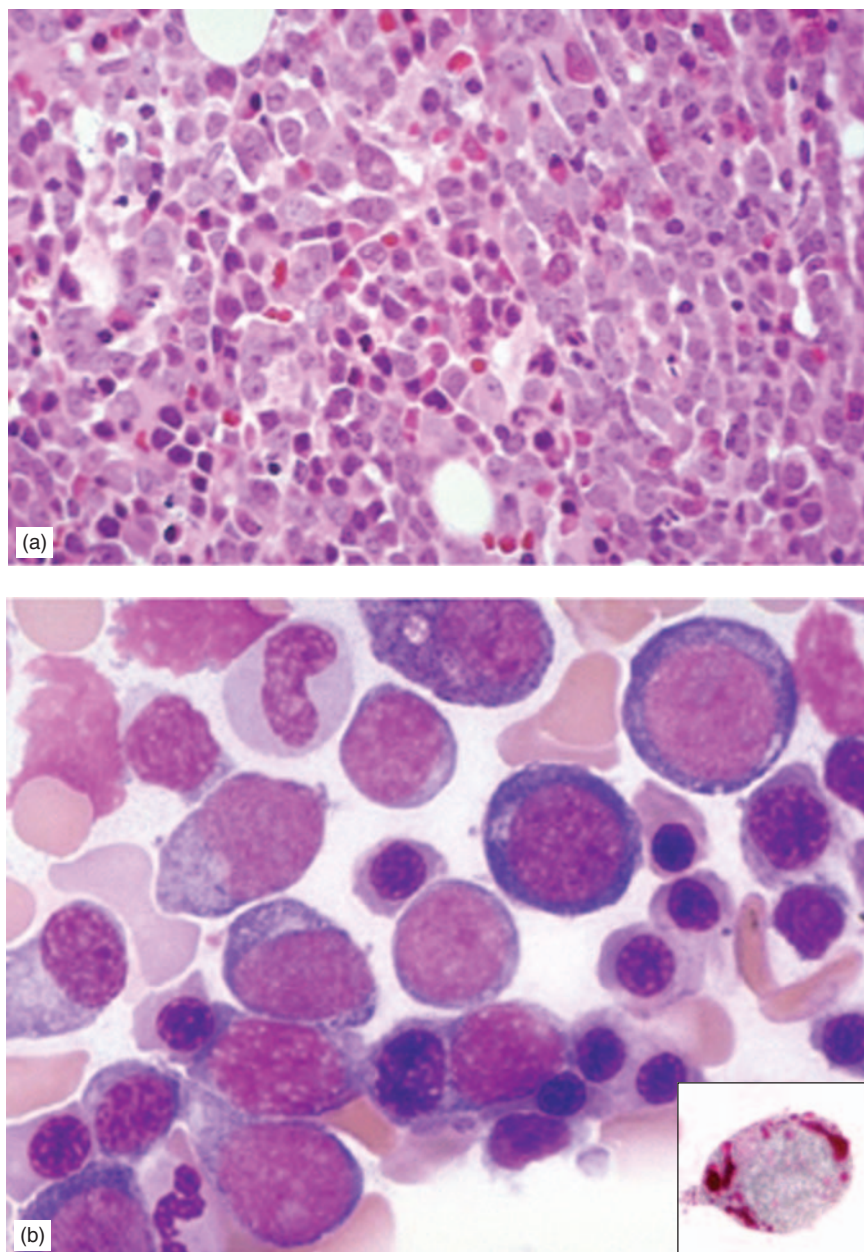


FIGURE 11.39 Erythroid/myeloid leukemia (erythroleukemia, AML-M6a). (a) Bone marrow biopsy section showing hypercellular marrow with increased immature cells. (b) Bone marrow smears show erythroid preponderance and left shift with increased myeloblasts. Inset shows an erythroid precursor with coarse PAS positive cytoplasmic granules.

In one report the average survival time for the AML-M6a was 30 months compared to 3 months for the AML-M6b [222].

Differential Diagnosis

The distinguishing feature of acute erythroleukemia (AML-M6a) from RAEB is the proportion of erythroid component in the bone marrow. In AML-M6a, 50% or more bone marrow nucleated cells are of erythroid lineage and $\geq 20\%$ of the non-erythroid population consists of myeloblasts. AML with multilineage dysplasia should also be included in the differential diagnosis. According to the WHO recommendation, if $\geq 50\%$ of the myeloid or megakaryocytic lineages show dysplasia, the case should be classified as AML with multilineage dysplasia.

Pure erythroid leukemia (AML-M6b) should be distinguished from megaloblastic anemia. The erythroid left shift and dysplastic changes are not so severe in megaloblastic anemia as in pure erythroid leukemia. Besides, in megaloblastic anemia, there is often evidence of vitamin B12 or folate deficiency, whereas serum levels of vitamin B12 and folate are normal or elevated in pure erythroid leukemia. The differential diagnosis of pure erythroid leukemia also includes ALL, minimally differentiated AML, AML without maturation, and AMKL.

Acute Megakaryoblastic Leukemia

Acute megakaryoblastic leukemia (AML-M7) is defined as an AML in which megakaryoblasts account for $\geq 50\%$ of the total blast cells [1]. Similar to the other types of AML, total

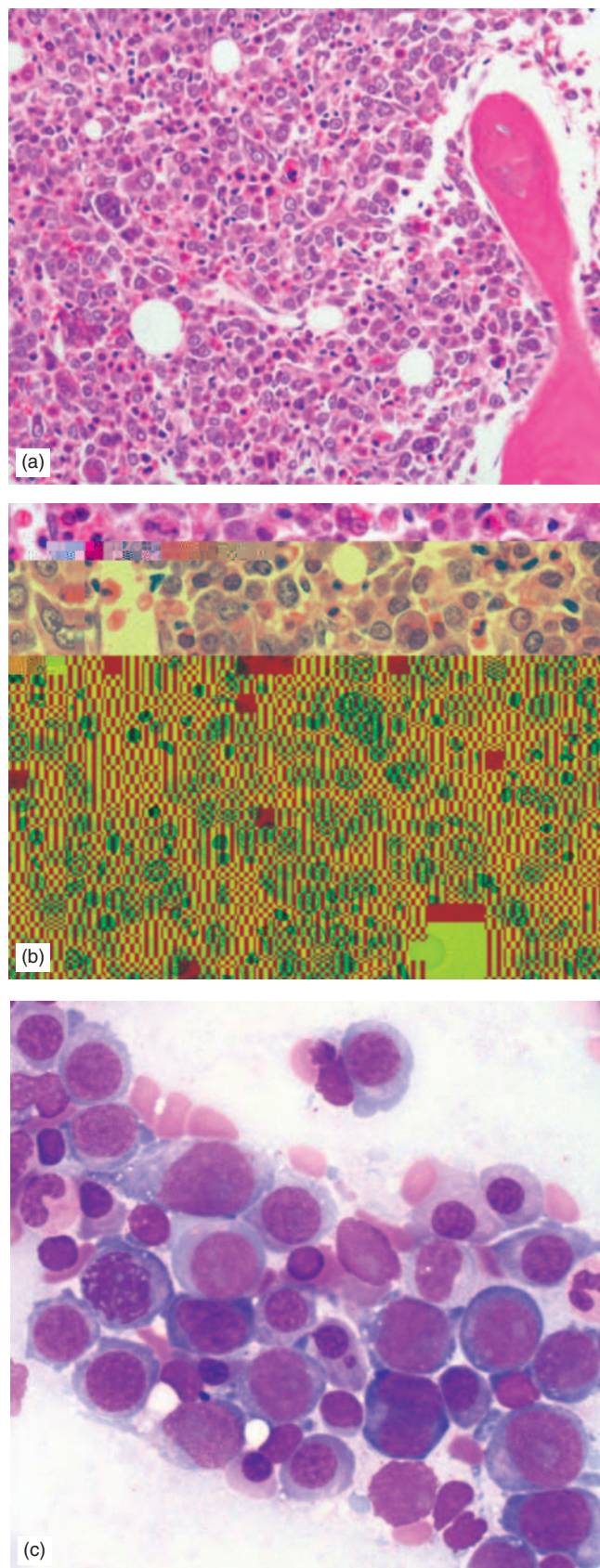


FIGURE 11.40 Pure erythroid leukemia (AML-M6b). Biopsy section reveals a markedly hypercellular marrow with sheets of blasts and immature erythroid cells: (a) low power and (b) high power. Bone marrow smear shows erythroid preponderance with increased erythroblasts (c).

blasts comprise $\geq 20\%$ of the bone marrow nucleated cells or peripheral blood differential counts.

Etiology and Pathogenesis

The etiology and pathogenesis of AMKL are not known. The strong association of Down syndrome with AMKL has raised the possibility that overexpression or mutation of certain hematopoietic regulatory genes located on chromosome 21 may be involved in the development of this leukemia. The strongest candidate gene for leukemogenesis is *RUNX1* (*AML1*) on chromosome 21q22.3. *RUNX1* is a transcription factor required for the production of all hematopoietic precursors. The translocation of the *RUNX1* gene is observed as t(8;21) in AML-M2, t(12;21) in childhood ALL, and t(3;21) in some cases of CML in blast crisis [223]. In addition, acquired mutations of the *GATA1* gene have been detected in the vast majority of patients with AML-M7 and Down syndrome. *GATA1* is located on the X chromosome (Xp11.23) and is a member of the transcription factor family that regulates proliferation and differentiation of hematopoietic cells [223].

Pathology

Morphology

Megakaryoblasts are the predominant component of the blast population in the bone marrow and/or peripheral blood (Figures 11.43 and 11.44) [1–4]. They are markedly pleomorphic, ranging from small, round cells with scanty cytoplasm and inconspicuous nucleoli, resembling hematogones, to large cells with abundant cytoplasm and prominent nucleoli. They often display cytoplasmic blebs or pseudopods and may appear in clusters mimicking metastatic tumors. Since megakaryoblasts may resemble hematogones, lymphoblasts, type 1 myeloblasts, or metastatic tumors and do not react with a specific cytochemical stain, often it is necessary to perform immunophenotypic and/or ultrastructural studies to be able to assign their megakaryocytic lineage.

The peripheral blood smears show circulating micro-megakaryocytes, megakaryocytic fragments, atypical giant platelets, and blasts. Red blood cells may show anisopoikilocytosis and scattered tear-drop shapes. Granulocytes may show dysplastic changes, such as hypogranulation and abnormal segmentation of their nuclei. Leukoerythroblastosis is uncommon.

Bone marrow fibrosis, and as a consequence, dry tap (failure of bone marrow aspiration) is a common feature. Bone marrow cellularity varies depending on the extent of fibrosis, but there is evidence of increased blast cells, either in clusters or as diffuse interstitial infiltration. There is also evidence of increased megakaryocytes with dysplastic morphology.

Immunophenotype and Cytochemical Stains

Megakaryoblasts carry one or more platelet glycoproteins (GP). These include CD41 (GPIIb/IIIa), CD42 (GPIb), and CD62 (GPIIIa), which are expressed both on the surface membrane and in the cytoplasm (Figure 11.44b) [224]. Therefore, both flow cytometry and immunohistochemical stains are helpful in their identification. Megakaryoblasts may also express CD36 and factor VIII. HLA-DR, CD45,

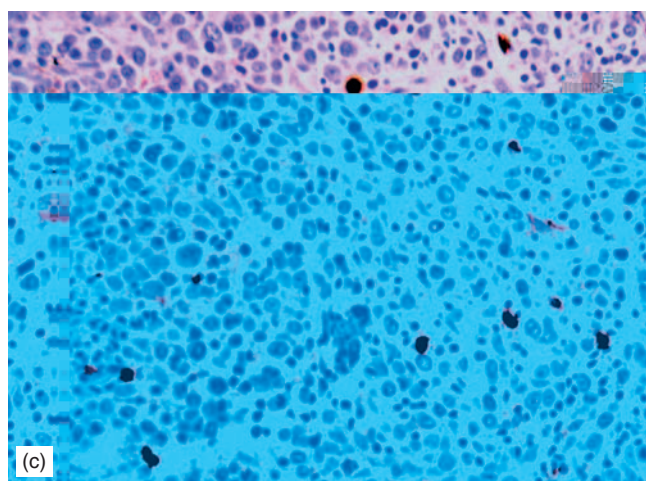
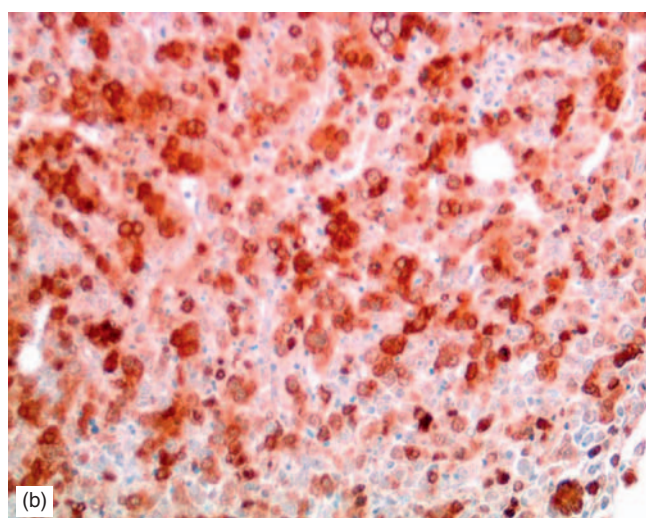
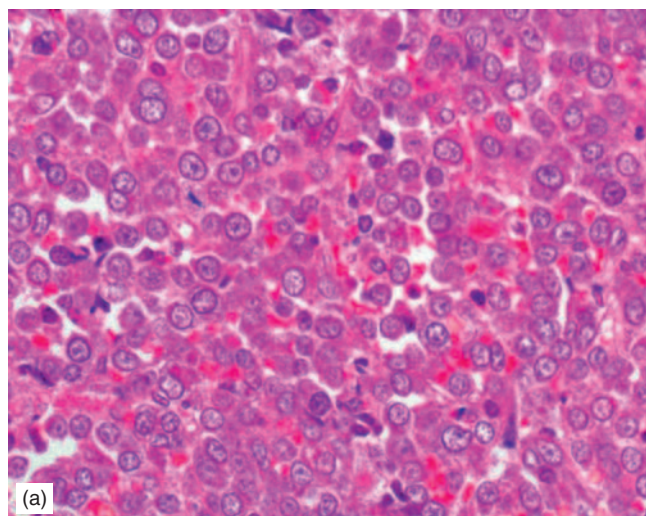


FIGURE 11.41 Pure erythroid leukemia (AML-M6b). Biopsy section reveals a markedly hypercellular marrow with sheets of blasts and immature cells (a). Immunohistochemical stain for hemoglobin A shows numerous positive cells (b). MPO stain is negative (c).

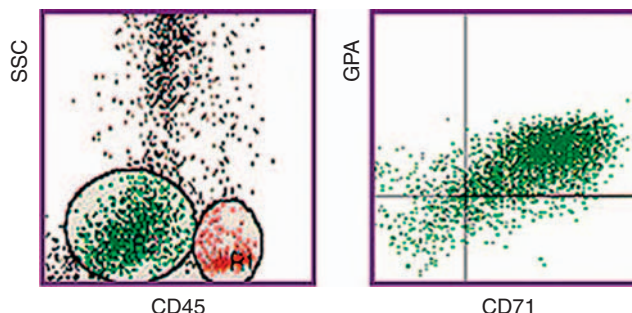


FIGURE 11.42 Pure erythroid leukemia (AML-M6b). Flow cytometry of the bone marrow cells shows a large population of CD45-negative cells (green gate) expressing CD71 and glycophorin A (GPA).

TdT, and lymphoid-associated markers are often negative, except for aberrant expression of CD7 which may be present in some cases [224, 225].

No conventional cytochemical stain is specific for megakaryoblasts. However, megakaryoblasts may show diffuse and/or globular PAS reaction and punctuate NSE staining. MPO and Sudan Black B are negative, but ultrastructural cytochemical staining or immunostaining reveals expression of platelet peroxidase. Megakaryoblasts also express acid phosphatase.

Molecular and Cytogenetic Studies

In addition to trisomy 21 in Down syndrome, $t(1;22)(p13;q13);(RBM15-MKL1)$ has also been reported in *de novo* AMKL (Figure 11.45) [226–228]. In addition to OTT-MAL transcript, there are reports of activated *JAK2* and *GATA1* mutations in AMKL [223, 229]. Chromosomal aberrations, such as $-7/7q-$, $-5/5q-$, and trisomy 8 have been reported particularly in the therapy-related type. Other chromosomal aberrations include $t(9;22)(q34;q11.2)$ and abnormalities of $3q21 \rightarrow q26$, $17q22$, $11q14 \rightarrow 21$, $21q21 \rightarrow 22$, and $16q22 \rightarrow 23$ [230–233]. Occasional cases of highly complex chromosomal abnormalities have been reported [234].

Clinical Aspects

AMKL represents 3–5% of the AMLs. It occurs in all ages with two distribution peaks: children between 1 and 3 years old and adults [235–238]. A significant proportion of affected children have Down syndrome [223, 238, 239]. In children with Down syndrome, the incidence of AML is 46-fold greater than that in the normal age group. AMKL accounts for at least 50% of the AML cases in patients with Down syndrome [234]. AMKL has also been associated with mediastinal germ cell tumors in young adult males. Hepatosplenomegaly is rare in adults but frequently observed in children, particularly in association with $t(1;22)$. This translocation has distinctive clinicopathologic features including onset in infancy, extensive bone marrow fibrosis with clustering of leukemic blasts mimicking metastatic tumors, and aggressive clinical course.

Differential Diagnosis

The differential diagnosis of AMKLs includes chronic idiopathic myelofibrosis, minimally differentiated AML,

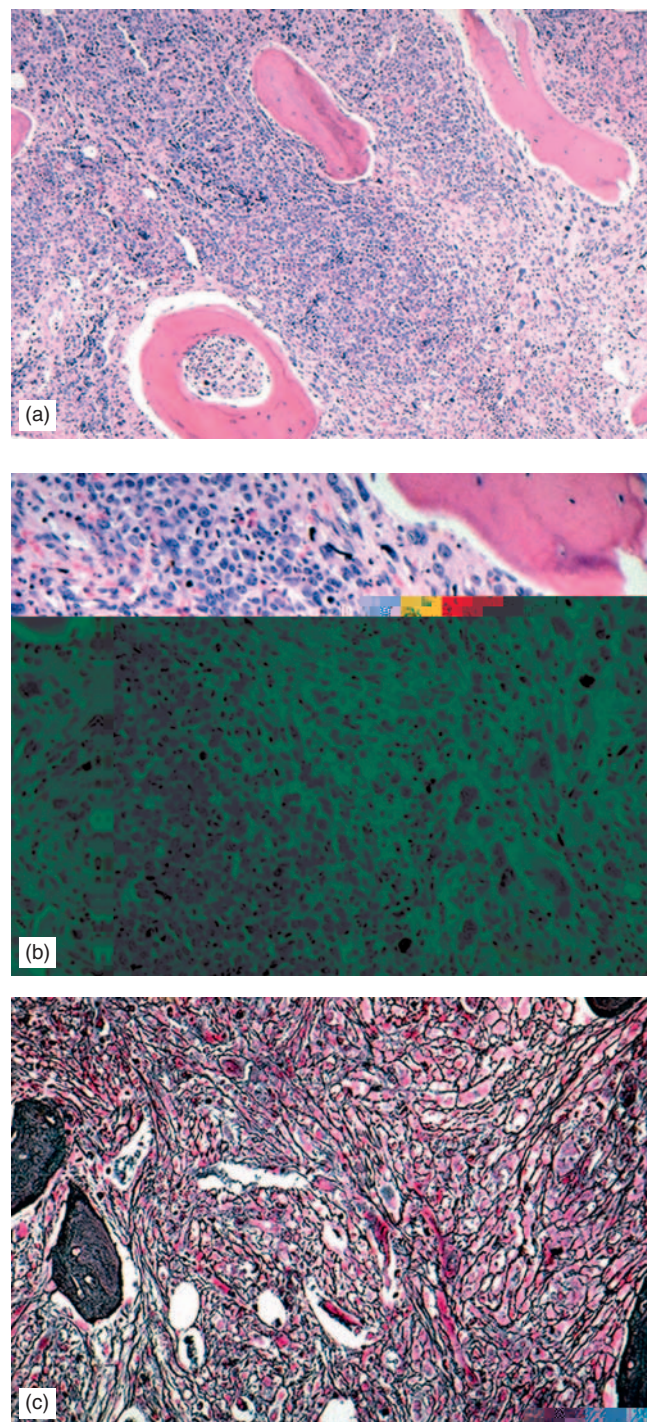


FIGURE 11.43 Acute megakaryoblastic leukemia. Bone marrow biopsy sections show large clusters of immature cells with areas of fibrosis and increased megakaryocytes: (a) low power and (b) high power. Reticulin stain reveals marked reticulin fibrosis (c).

AML without maturation, ALL, APMF, and metastatic tumors.

The presence of megakaryoblastic clusters embedded in the fibrotic bone marrow may mimic metastatic carcinoma, non-Hodgkin lymphoma, neuroblastoma, or rhabdomyosarcoma.

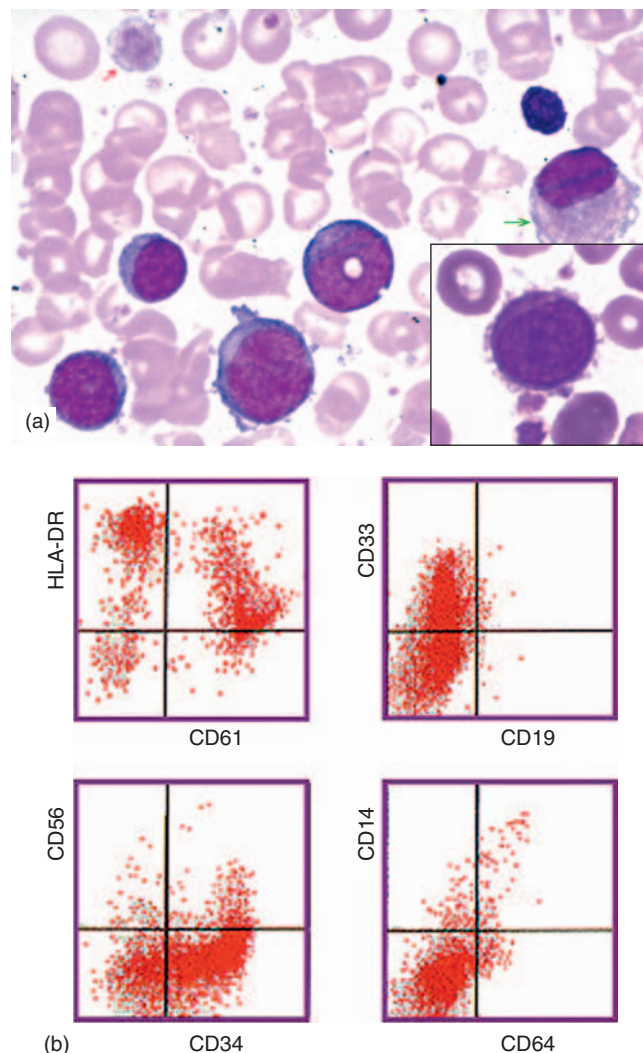


FIGURE 11.44 Acute megakaryoblastic leukemia. (a) Blood smear demonstrating numerous blast cells of various sizes, some with cytoplasmic blebs. A micromegakaryocyte (green arrow) and a giant platelet (red arrow) are present. (b) Flow cytometry of peripheral blood demonstrates a population of cells expressing CD34 and CD61 consistent with megakaryoblasts.

The diagnosis of AMKL is suggested by certain clinico-pathological features, such as Down syndrome, myelofibrosis with no or minimal leukoerythroblastosis, presence of megakaryoblasts in the peripheral blood, and/or evidence of $t(1;22)$. The diagnosis is confirmed when at least 50% of the total blast population is of megakaryocytic origin evidenced by the presence of platelet myeloperoxidase (PPO) by electron microscopy and/or expression of CD41, CD42, CD61, and factor VIII.

Acute Basophilic Leukemia

Acute basophilic leukemia is a rare type of AML with basophilic differentiation [1, 240–242]. ABL is either *de novo* or the result of basophilic blast transformation in CML.

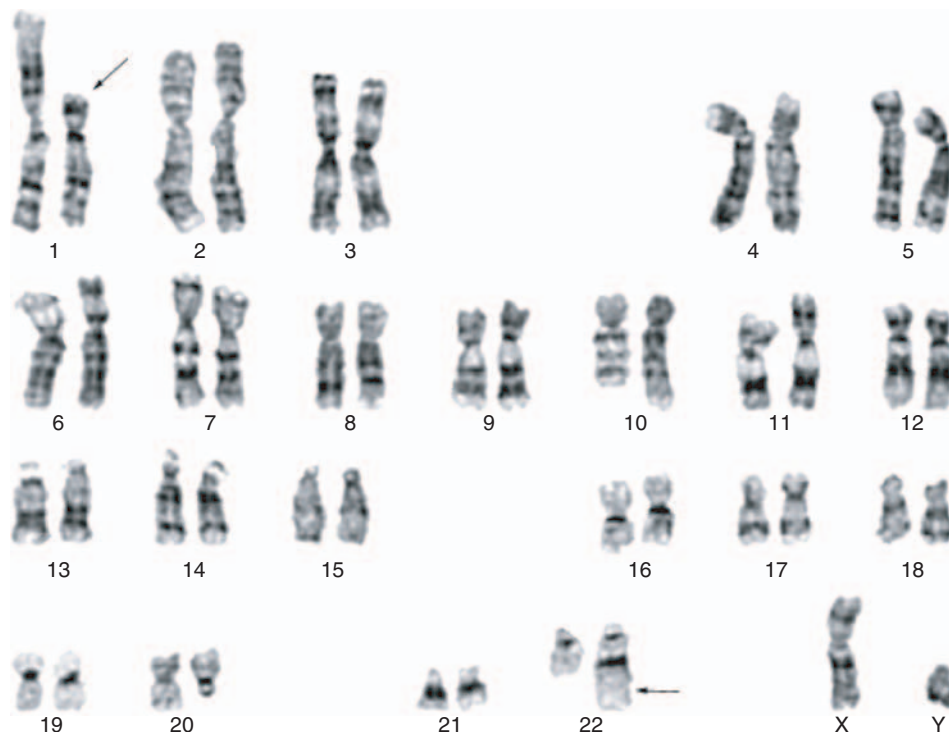


FIGURE 11.45 Acute megakaryoblastic leukemia. G-banded karyotype demonstrating t(1;22).

Etiology and Pathogenesis

The etiology and pathogenesis of ABL are not known.

Pathology

Morphology

The blasts show a high nuclear:cytoplasmic ratio with variable amounts of cytoplasm containing coarse basophilic granules. The nuclei are round, oval, or bilobed with a fine chromatin and one or more prominent nucleoli. Scattered mature basophils are often present. Electron microscopy reveals characteristic features of basophilic granules [1].

Bone marrow biopsy sections appear hypercellular with large clusters or sheets of blast cells and marked reduction in the normal hematopoietic components. There may be dysplastic erythropoiesis in the bone marrow. A variable degree of reticulin fibrosis is often present, particularly in advanced stages of the disease.

Blood smears usually show the presence of blasts with basophilic granules and mature basophils. Anisopoikilocytosis and thrombocytopenia may be present.

Immunophenotype and Cytochemistry

The blast cells express some of the myeloid-associated markers, such as CD13, CD15, and CD33. They are often positive for CD34 and HLA-DR and may express CD9, CD25, CD117, or CD203c [240, 243]. The cytoplasmic basophilic granules may stain with toluidine blue but are usually negative for MPO and NSE.

Molecular and Cytogenetic Studies

No specific chromosomal aberration is known for ABL. There are sporadic reports of abnormalities of deletion of

12p, t(6;9)(p23;q34), t(8;21)(q22;q22), and t(X;6)(p11;q23) [244–246]. Cases of basophilic transformation in CML show t(9;22)(q34;q11.2).

Clinical Aspects

Acute basophilic leukemia is a rare disease probably representing 1% of all AMLs. It occurs at any age including infancy. Clinical findings may include anemia, skin rashes, hepatosplenomegaly, and gastric ulcers.

Differential Diagnosis

Differential diagnosis includes blast transformation of CML, certain subtypes of AML with basophilia, particularly the ones associated with t(6;9) or abnormalities of 12p, and occasional cases of ALL with coarse azurophilic granules.

Acute Panmyelosis with Myelofibrosis

Previously referred to as *acute myelofibrosis*, *acute myelofibrosis*, or *acute myelodysplasia with myelofibrosis*, APMF is an acute leukemia with panmyeloid proliferation, increased blasts, and bone marrow fibrosis [1, 247, 248]. This entity shares significant overlapping features with AMKL, such as bone marrow fibrosis, unascrable marrow, dysplastic megakaryocytes, and the presence of megakaryoblasts. However, in AMKL, megakaryoblasts comprise the predominant proportion of the blast population, whereas in APMF majority of the blasts are of non-megakaryocytic origin [1, 249].

Etiology and Pathogenesis

The etiology and pathogenesis of APMF are not known.

Pathology

Morphology

Bone marrow fibrosis is one of the morphologic hallmarks. Marrow fibrosis often leads to unaspirable bone marrow (dry tap) and inadequate marrow smears. The bone marrow cellularity varies depending on the extent of fibrosis. There is evidence of increased blast cells, either in clusters or as diffuse interstitial infiltration [1, 248, 249]. There is also evidence of increased megakaryocytes with dysplastic morphology, including micromegakaryocytes and nonlobulated and/or hypolobulated forms.

The peripheral blood smears may show absent to mild anisopoikilocytosis with macrocytes and occasional teardrop shapes. Granulocytes may show dysplastic changes, such as hypogranulation and abnormal segmentation of their nuclei. Abnormal platelets may be present. Circulating blasts are variable, ranging from a few to a frank leukemic picture [1, 248].

Immunophenotype and Cytochemical Stains

The majority of the blast cells express CD34, often with one or more myeloid-associated markers, such as CD13, CD33, and/or CD117 and cytoplasmic MPO [250]. Only a minority of blast cells express platelet-associated molecules, such as CD41, CD42, or CD61. Cytochemical stains may show presence of MPO-positive blast cells.

Molecular and Cytogenetic Studies

Monosomy 7 and deletion of 5q or 7q are the most frequent chromosomal aberrations in this condition. Also, interstitial deletion of the long arm of chromosome 11 has been reported [250, 251].

Clinical Aspects

APMF is a rare type of AML often presenting with marked cytopenia. Splenomegaly is minimal or absent. It has an unfavorable prognosis with a median survival of <1 year in some reports [248, 251].

Differential Diagnosis

As mentioned earlier, this entity shares many overlapping features with AMKL, such as bone marrow fibrosis, unaspirable marrow, dysplastic megakaryocytes, and the presence of megakaryoblasts. However, unlike AMKL, in APMF the majority of the blasts are of non-megakaryocytic origin. The differential diagnosis also includes chronic idiopathic myelofibrosis, minimally differentiated AML, AML without maturation, ALL, and metastatic tumors.

Granulocytic Sarcoma

Granulocytic sarcoma (chloroma) refers to extramedullary tumors of myeloid precursors. The term “chloroma” was originally used in response to the green color of the tumor due to the presence of MPO in the tumor cells. Granulocytic sarcoma is associated with CML, CML in blast crisis, and *de novo* AML. It either develops during the active phase of

the disease or represents relapse without evidence of recurrent disease in the blood or the bone marrow. The most frequent sites of involvement include skin, lymph nodes, respiratory system, gastrointestinal tract, CNS, and subperiosteal structures of the skull, ribs, vertebrae, and pelvis.

Granulocytic sarcomas are composed of various proportions of immature and mature myeloid cells (Figure 11.46). Some tumors resemble CML and consist predominantly of mature granulocytic cells, and others similar to AML show the predominance of myeloblasts and immature myeloid cells. The ones with blasts and immature cells may resemble lymphomas or non-hematopoietic malignancies. The presence of immature eosinophils is a distinctive feature of granulocytic sarcomas. Tumor cells in these lesions express myeloid-associated molecules in the biopsy sections, such as MPO, NES, and/or lysozyme.

OTHER TYPES OF AML

Acute Myeloid Leukemia With Chromosome 3 Aberrations and Thrombocytosis

Approximately 3% of AML cases show cytogenetic abnormalities of 3q along with thrombocytosis. The cytogenetic aberrations include the *inv*(3)(q21q26), *t*(3:3)(q21;q26), and *t*(5:3)(q14;q21q26). In some cases thrombocytosis may exceed 1,000,000/ μ L. Bone marrow samples consistently show increased numbers of megakaryocytes, including micromegakaryocytes.

Other Recurrent Genetic Abnormalities

Other recurrent genetic abnormalities associated with AML include *t*(1;22)(p13;q13) (*RBM15;MKL1*), *t*(9;22)(q34;q11.2) (*BCR;ABL1*), AML with C/EBP α mutation, and AML with mutated nucleoplasmin (*NPM*) gene. The *t*(9;22) is seen in CML in blast transformation, but less frequently may be present *de novo* without a history of CML.

The *t*(1;22), as mentioned earlier, has distinctive clinicopathologic features including onset in infancy, extensive bone marrow fibrosis with clustering of leukemic blasts (usually megakaryoblasts) mimicking metastatic tumors, and aggressive clinical course [252–254].

C/EBP α (CCAAT/enhancer-binding protein alpha) is a critical regulator for early myeloid differentiation. Mutations in C/EBP α occur in 10% of patients with AML, leading to the expression of a 30 kDa dominant-negative isoform (C/EBP α 30) [255–257].

The mutations of the *NPM* gene result in aberrant cytoplasmic localization of the NPM protein (NPMc+). These mutations occur in 25–35% of adult AML that show normal karyotype [258–260]. Patients with NPM mutations show high remission induction rates and improved survival [259].

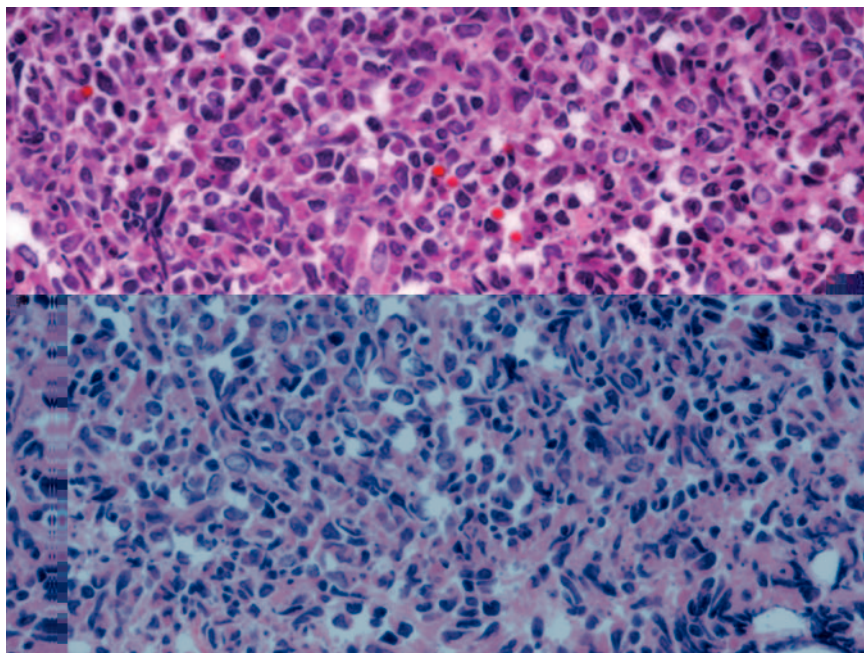
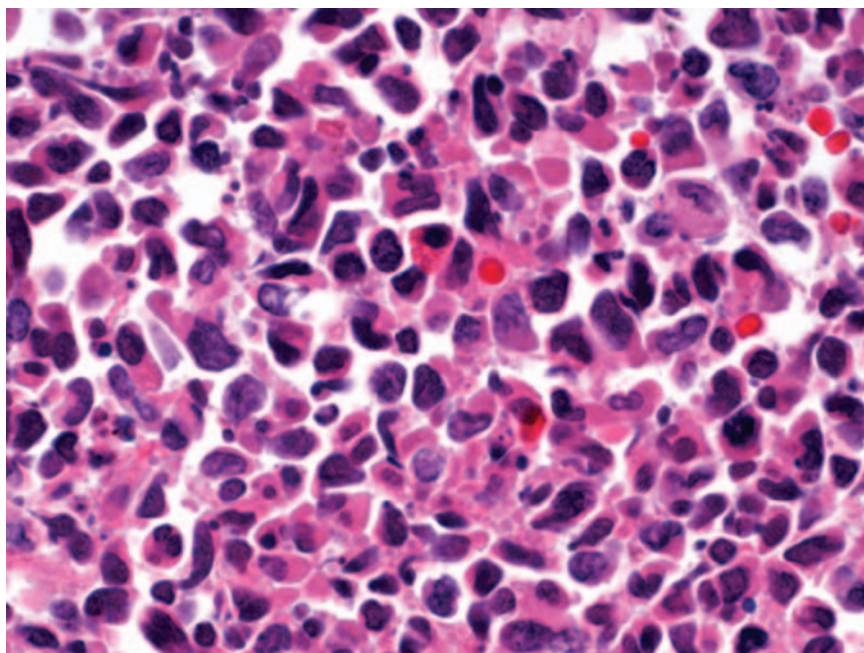


FIGURE 11.46 Soft tissue infiltration of immature myeloid cells representing granulocytic sarcoma (chloroma).



Hypoplastic Acute Leukemia

Hypoplastic acute leukemia, or hypocellular acute leukemia, is a leukemic condition characterized by marked bone marrow hypocellularity. The etiology and pathogenesis of this leukemia are not known, but bone marrow toxicity may play a contributing role. In a study by Gladson and Naeim [261], a history of alcohol abuse was demonstrated in 30% of the patients, potential exposure to toxic chemicals in 20%, and history of chemotherapy or radiation therapy due to a second malignancy in 20%. This type of leukemia tends to involve elderly people, primarily men.

The bone marrow is hypocellular, usually $<30\%$ of the average cellularity in the normal matching age group. Blast cells are prominent and account for $\geq 20\%$ of the bone marrow cells (Figure 11.47). They display scant-to-moderate amounts of cytoplasm, round or oval nuclei with fine nuclear chromatin, and one or more prominent nucleoli. In most studies, blast cells appear to be of myeloid origin by the presence of cytoplasmic azurophilic granules, Auer rods, positive reactions for MPO, Sudan Black B, or expression of myeloid-associated molecules, such as CD13, CD33, and CD117.

The differential diagnoses include aplastic anemia and hypocellular MDS. Diagnosis is made based on the presence of $\geq 20\%$ blasts in a hypocellular marrow.

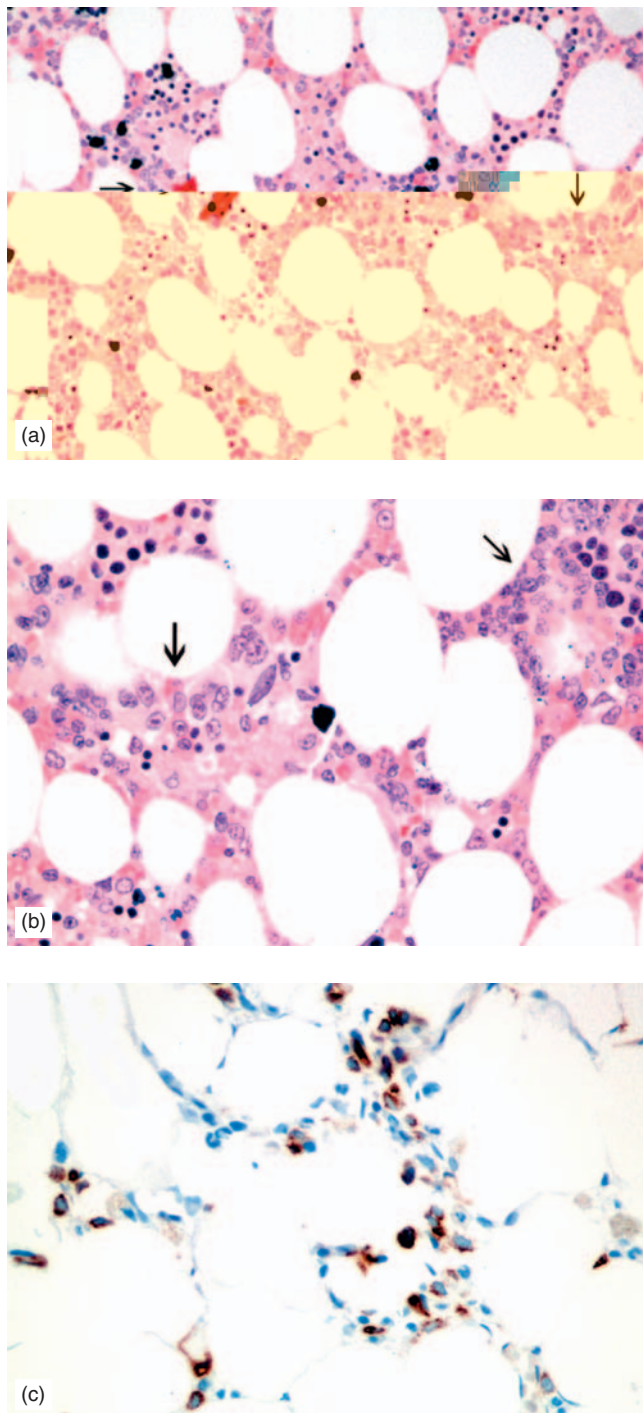


FIGURE 11.47 Hypoplastic acute myeloid leukemia. Bone marrow biopsy section showing hypocellularity and increased blasts (a, low power; b, high power). Immunohistochemical stain for CD34 reveals numerous positive cells (c).

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The Neoplasms of Precursor Lymphoblasts

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Lymphoblastic neoplasms represent leukemias or lymphomas of precursor lymphoid cells – blast cells committed to lymphoid differentiation. Clinically, they are divided into two major categories: lymphoblastic lymphoma (LBL) and acute lymphoblastic leukemia (ALL). Typically, LBL represents a neoplastic process involving extramedullary lymphoid tissues with $\leq 25\%$ bone marrow involvement, and ALL involves $>25\%$ of bone marrow with or without extramedullary lesions [1, 2]. Since LBL and ALL share considerable clinicopathological features and present similar biological properties, they are considered the same disease. In the WHO classification, LBL and ALL are lumped together as precursor lymphoid neoplasms and are divided into two major categories: (1) precursor B-lymphoblastic leukemia/lymphoma and (2) precursor T-lymphoblastic leukemia/lymphoma [1]. In addition to these two categories, there are rare types of precursor acute lymphoblastic leukemia/lymphoma, such as NK-cell type, granular ALL, hypoplastic ALL, and ALL with eosinophilia, which are briefly discussed at the end of this chapter.

PRECURSOR B-LYMPHOBLASTIC LEUKEMIA/LYMPHOMA

Precursor B-lymphoblastic leukemia/lymphoma (B-ALL/B-LBL) may initially present itself as ALL with the involvement of bone marrow and/or blood, or LBL with the involvement of the lymphoid and/or other extramedullary tissues. In a significant proportion of the cases, however, both bone marrow and extramedullary tissues are

involved. ALL and LBL are regarded as different clinical presentations of the same disease.

Etiology and Pathogenesis

The etiology and pathogenesis of B-ALL and B-LBL are not clearly understood. Epidemiological studies suggest that exposure to ionizing radiation, certain chemicals, viruses or bacteria, or other environmental factors may play a role in the development of some subcategories of B-ALL/B-LBL [3–6]. For example, studies of children with precursor B-ALL show that the affected children had limited social contacts during their infancy, or had not received certain vaccinations, particularly for *Haemophilus influenzae* [7]. Chromosomal instability syndromes, such as Fanconi anemia and Ataxia-telangiectasia, are considered risk factors [8–11].

The common chromosome translocations in precursor B-ALL often arise prenatally and appear to play a role in the pathogenesis of the disease [12–15]. For example, $t(12;21)(p13;q22)$ fuses the *ETV6* and *AML1* genes together. The breakpoints in the *ETV6* and *AML1* genes occur randomly, meaning that each patient's leukemic cells have a unique breakpoint in the DNA sequence [12, 13]. Analyses of pairs of identical twins with concordant acute lymphoblastic leukemia and $t(12;22)$ have shown that leukemic cells from both twins share identical breakpoints in the *ETV6* and *AML1* genes. The assumption is that in these twins a preleukemic clone with $t(12;22)$ has been developed, which after birth has transformed into a full-blown leukemia [7]. For this transformation to occur, there may be a need for some additional postnatal events supporting the

“two-hit” model of leukemogenesis for the development of B-ALL [14, 15].

Chromosomal translocations result in the development of chimeric genes with fusion protein products. These products may play an important role in the development of leukemia. For example, *BCR/ABL1* fusion gene products of t(9;22) in precursor B-ALL patients affect the RAS/MAPK pathway of signal transduction and promote leukemogenesis (see later).

Pathology

Morphology

The bone marrow biopsy and clot sections are usually hypercellular and are diffusely infiltrated by sheets of uniformly appearing blast cells [1, 2, 16, 17]. These cells have scanty basophilic cytoplasm with round, oval, or indented nuclei; finely dispersed nuclear chromatin; and prominent or

indistinct nucleoli. In some cases, the leukemic blasts may appear pleomorphic with variable amounts of cytoplasm or may show convoluted nuclei. Mitotic figures are variable, but often easily detectable. Bone marrow fibrosis and osteoporosis are sometimes present. Fibrosis may be mild, extensive, focal, or diffuse, and it may lead to unsuccessful bone marrow aspiration (dry tap). It is more frequent in the B-ALL than in the T-ALL. Bone marrow necrosis may be present in some cases. Necrosis is usually of the coagulative type with the preservation of the basic outline of the necrotic cells.

Bone marrow smears and touch preparations show numerous blast cells, which are often small with scanty non-granular blue cytoplasm, fine chromatin, and indistinct nuclei (Figure 12.1). But less frequently, the blast cells are larger and more pleomorphic and show variable amounts of cytoplasm which may display vacuolization or azurophilic granules (5–10% of the cases), and one or more prominent nucleoli. Some cases may show cytoplasmic tails (pseudopods) referred to

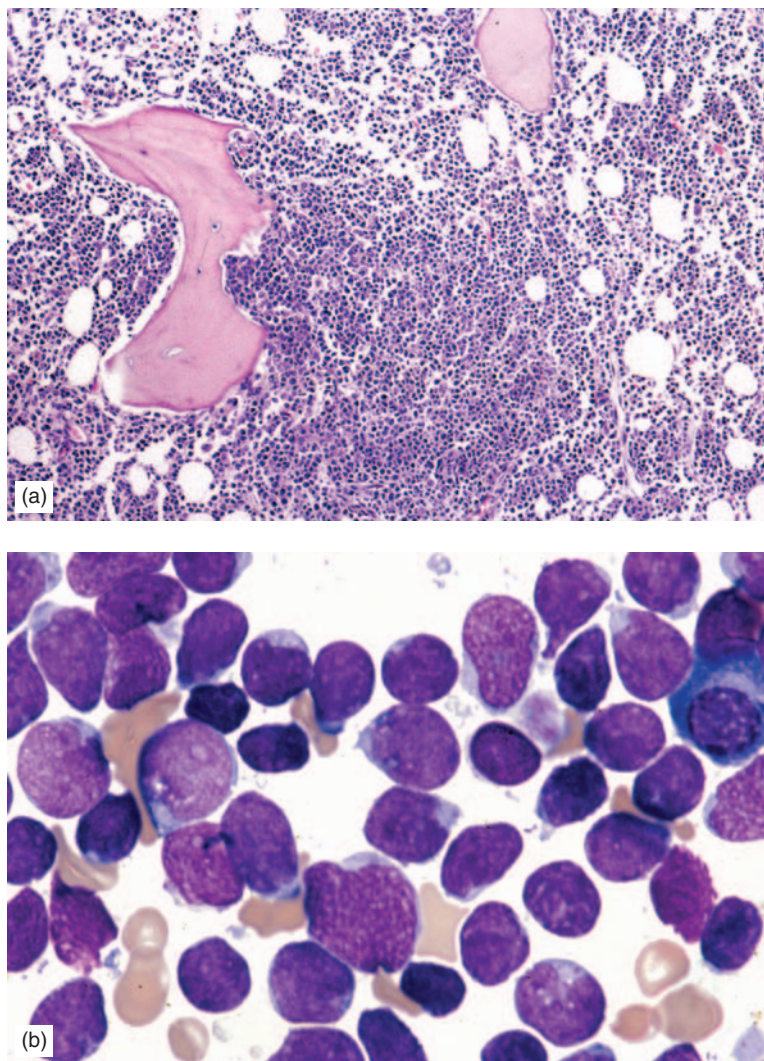


FIGURE 12.1 Bone marrow biopsy section (a) and bone marrow smear (b) of a patient with acute lymphoblastic leukemia.

as *hand-mirror* cells (Figure 12.2). In the case of bone marrow necrosis, the aspirated necrotic cells appear as a mixture of smudge cells, bare degenerated nuclei, and cell debris with an increased amorphous, basophilic background material.

Lymphoblasts may also be present in the peripheral blood smears in variable numbers (Figure 12.1b). They account for the majority of leukocytes in patients with $WBC > 10,000/\mu L$. Approximately 20% of patients at the time of diagnosis present with a leukocyte count exceeding $50,000/\mu L$. Anemia, granulocytopenia, and/or thrombocytopenia are common features.

The involvement of lymph nodes and other tissues is usually diffuse with total or partial effacement of the normal architecture and morphologic features similar to those described earlier in the bone marrow biopsy sections.

Immunophenotype and Cytochemical Stains

The precursor B-lymphoblasts characteristically express nuclear terminal deoxynucleotidyl transferase (TdT) and CD79a, are weakly positive for CD45, and lack the expression of surface membrane immunoglobulin (SIg) [1, 2, 18]. These cells are divided into three phenotypic subcategories [2] (Table 12.1):

- Early precursor B
- Intermediate precursor B (representing common ALL)
- Late precursor B (representing pre-B-ALL).

The early precursor B-cells express cytoplasmic CD22, HLA-DR, and usually CD19. The leukemia cells of intermediate precursor B-cells (common ALL) are positive

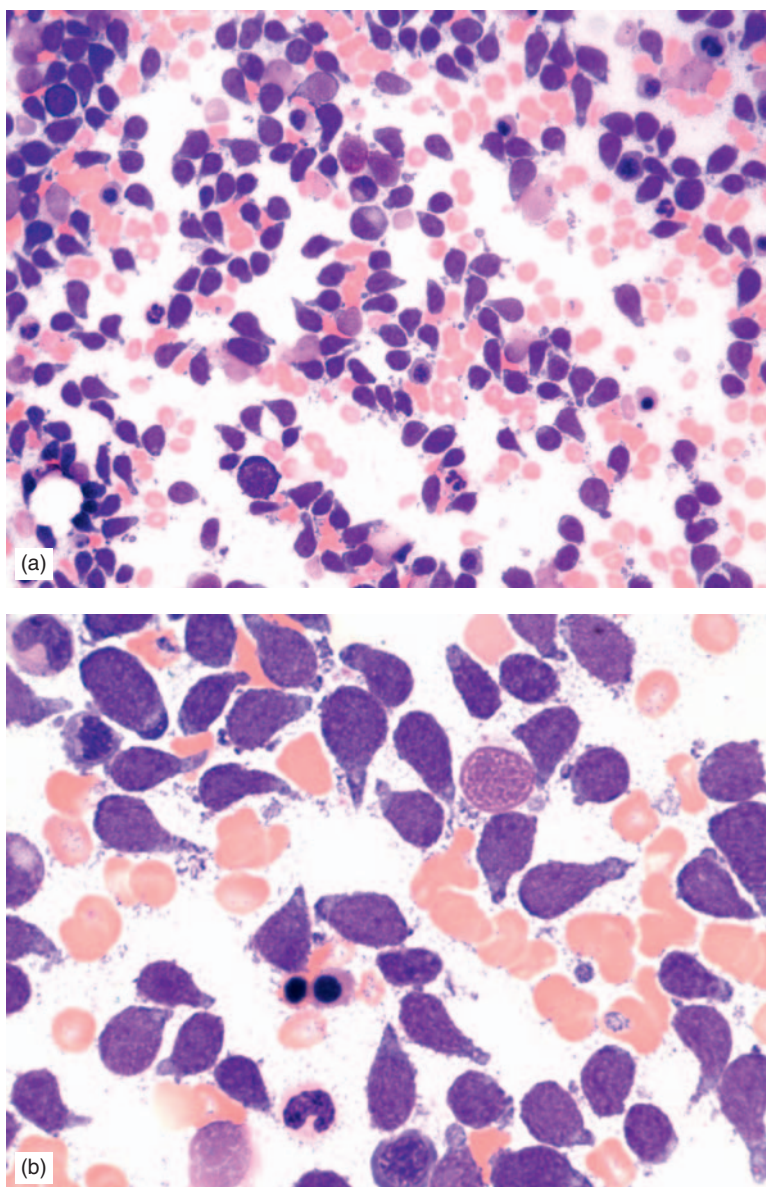


FIGURE 12.2 Bone marrow smear demonstrating the hand-mirror variant of acute lymphoblastic leukemia: (a) low power and (b) high power.

for CD10 (Figure 12.3), and cells representing the late precursor B-ALL are CD20-positive and express cytoplasmic μ heavy chain (Table 12.1).

Expression of CD34 has been observed in about 40% of the cases of B-ALL, particularly in the early and intermediate precursor categories. Aberrant expression of myeloid markers, such as CD13, CD15, CD33, and CD68, has been observed in some cases of B-ALL, particularly in association with certain chromosome translocations. For example, blast cells in patients with t(12;21) may show coexpression of CD13 and/or CD33, and leukemic cells in cases with t(4;11) and 11q23 abnormalities may show coexpression of CD15 and/or CD68.

Precursor B-cells are negative for MPO but may show coarse PAS-positive cytoplasmic granules. In some cases blast cells may show punctuate or focal positive reaction for NSE, or light gray staining for Sudan Black B stain.

Cytogenetic and Molecular Studies

Cytogenetic abnormalities in B-ALL/B-LBL are considered among the most useful prognostic indicators. They are often associated with distinct immunophenotypic features. Reciprocal chromosomal translocations, hyperdiploidy, and hypodiploidy are the most common cytogenetic abnormalities in ALL/LBL patients [19–25] (Table 12.2). The major recurrent

chromosomal translocations include t(9;22) (Philadelphia chromosome-positive ALL), t(4;11), t(1;19), and t(12;21).

Philadelphia Chromosome, t(9;22)(q34;q11.2)

This abnormality is observed in about 5% of children and 20% of adults with B-ALL/B-LBL [22, 25–27]. It is the most frequent rearrangement in adult ALL. Molecular studies, such as reverse transcriptase polymerase chain reaction (RT-PCR), for the detection of *BCR/ABL1* rearrangement should be used when there is non-diagnostic cytogenetic analysis, and for monitoring patients under therapy in a more quantitative manner. Over 50% of ALL patients with Philadelphia chromosome (*Pb¹*) have additional chromosomal abnormalities such as monosomy 7. *Pb¹* positivity is associated with poor prognosis. For example, the likelihood of remaining in remission for 3 years has been reported to be 17% for adult patients with *Pb¹* compared to 48% in patients with no *Pb¹* [22, 28].

Molecular studies of *BCR/ABL1* fusion in t(9;22) reveal two distinct subgroups giving rise to two types of fusion proteins weighing 185–190 and 210 kDa [22, 29–31].

TABLE 12.1 Immunophenotypic characteristics of precursor B-lymphoblastic leukemia/lymphoma.

Stage	Immunophenotype
Early precursor	HLA-DR, TdT, cCD22, CD79a, CD19
Intermediate precursor (common)	HLA-DR, TdT, cCD22, CD79a, CD19, CD10, CD20 (variable)
Late precursor	HLA-DR, TdT (variable), cCD22, CD79a, CD19, CD10, CD20, cytoplasmic μ

TABLE 12.2 Cytogenetic abnormalities and prognosis in precursor B-lymphoblastic neoplasms.

Cytogenetics	Genes	Frequency	Prognosis
t(9;22)(q34;q11.2)	<i>BCR/ABL1</i>	25%, adults 3–5%, children	Poor
t(4;11)(q21;q23)	<i>AF4/MLL*</i>	5%, children	Poor
t(1;19)(q23;p13.3)	<i>PBX1**/E2A</i>	6%, children	Poor
t(12;21)(p13;q22)	<i>ETV6(TEL)/AML1</i>	25%, children 3–4%, adults	Favorable
Hyperdiploidy		20–25%	Favorable
Hypodiploidy		5%	Poor

Alternative designations:
* *ALL* or *HRX*.
** *PRL*.

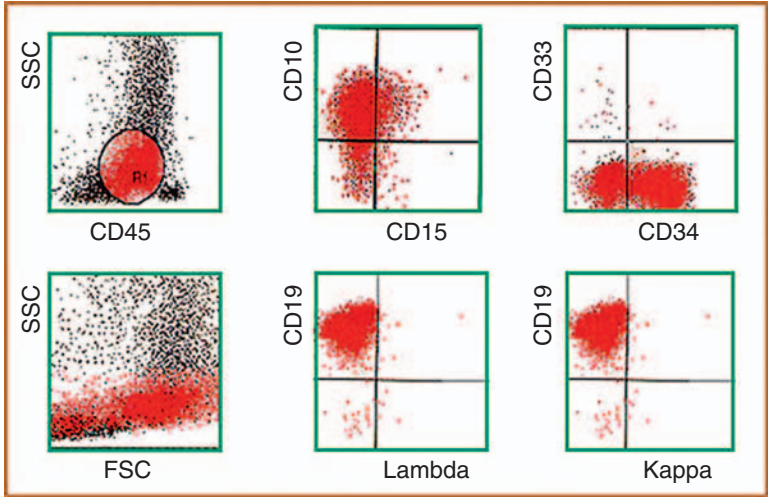


FIGURE 12.3 Bone marrow flow cytometric study of a patient with precursor B-acute lymphoblastic leukemia. The blast cells are dimly CD45-positive and express CD10, CD19, and CD34.

Approximately 30–50% of adult ALL patients show *BCR-ABL1* rearrangement similar to that observed in chronic myelogenous leukemia (CML). The translocation breaks occur within the major-breakpoint cluster region (referred to as M-bcr) of the *BCR* gene and the *ABL1* gene, leading to a chimeric gene which encodes for a 210-kDa fusion protein (p210) [22]. In children and approximately 50–70% of adults with t(9;22), the breakpoint occurs further downstream in the *BCR* gene, referred to as the minor bcr (m-bcr). This fusion gene encodes smaller fusion proteins ranging from 185 to 190 kDa (p185) [22].

Both fusion proteins, p185 and p210, exhibit tyrosine kinase activity similar to that of the native *ABL*-encoded enzyme, but at a higher level. In addition, the native protein is found in both the nucleus and the cytoplasm, whereas the fusion protein is exclusively cytoplasmic. As in CML, these proteins are appealing candidates for molecular-targeted therapies (e.g. imatinib).

At the molecular level, the *BCR-ABL1* fusion event can be detected in a qualitative manner by Southern blot or quantitatively and extremely sensitively by real-time RT-PCR. The standard Southern blot procedure employs hybridization with a radiolabeled or chemiluminescent *BCR* probe. If the translocation has occurred, novel bands or junction fragments will be observed in addition to the unarranged germline bands when informative restriction endonucleases are used (Figure 12.4). It is customary to run two lanes, with two different restriction enzymes, since apparent rearrangement with just one could be due to a benign polymorphism (RFLP) rather than a true translocation.

More recently, the cumbersome Southern blot procedure has been largely replaced by PCR methods because of their much greater efficiency, quantitative accuracy, and sensitivity. However, the Southern blot can easily target large stretches of DNA, whereas the expanse of the breakpoint cluster region in ALL (as well as in CML) is too large to be covered reliably by a DNA-targeted primer set. Instead, the target chosen is the *BCR-ABL1* fusion transcript from which long introns have been spliced out to yield a target of more

manageable size [32]. Naturally, this RNA-based test requires a reverse transcriptase (RT) step in order to generate a DNA target which can then be amplified by PCR. This introduces a potential confounding variable in the assay, owing to the lability of RNA. Blood or bone marrow specimens collected for this test must reach the laboratory in an expeditious manner (in our laboratory, we reject specimens that are >48 h old) and should begin RNA extraction and processing immediately.

Moreover, the *BCR-ABL1* quantity detected is typically given in relative terms, compared to the mRNA of a standard “housekeeping” gene such as *G6PDH*. Sensitivities of *BCR-ABL1* mRNA per 10,000 or 100,000 control gene mRNAs allow a rough extrapolation of the number of leukemic cells relative to the normal cells in the specimen (based on the assumption – which may not always be true – that the stability of the two transcripts is roughly equal). Sensitivities at this level, assuming that they are accurate and reproducible, are suitable for the detection of minimal residual disease in treated patients, and monitoring of tumor loads over long-term therapy. However, caution must be exercised in the setup of this assay so that it is not *too* sensitive, since *BCR-ABL1* fusion events have been detected in blood and tissues of healthy individuals, including children [33]. In our laboratory, we are skeptical of any apparently positive result that does not appear or reach its logarithmic “crossing point” until after PCR cycle number 40 or 45 (Figure 12.5).

t(4;11)(q21;q23)

This translocation, which results in a chimeric fusion gene, *AF4/MLL* is observed in about 5% of patients with ALL (Figure 12.6) [34, 35]. The characteristic clinicopathological features associated with t(4;11) are [22, 36–39]:

- High leukocyte count, often $\geq 200,000/\mu\text{L}$
- Early precursor B-cell type with lack of expression of CD10 and CD20 (Table 12.1)
- Frequent coexpression of myeloid-associated markers CD15 and CD65 (Figure 12.7)
- Poor prognosis.

In t(4;11)(q21;q23), the *AF4* gene on chromosome 4 fuses to the *MLL* (or *ALL1*) gene on chromosome 11 (Figures 12.8 and 12.9) [34, 35]. The *AF4/MLL* fusion gene is transcribed into a hybrid mRNA. This transcript can be detected by RT-PCR techniques for establishing the diagnosis or monitoring the residual disease.

The majority of infants with ALL have t(4;11)(q21;q23) or other abnormalities of 11q23.

t(1;19)(q23;p13.3)

This translocation is primarily seen in childhood pre-B-ALL (late precursor B) with a rate ranging from 6% to 30% in various reports [22, 40–43]. The t(1;19) is less frequent in adult pre-B-ALL [22]. Two forms of t(1;19) have been reported: a reciprocal translocation and an unbalanced form as der(19)t(1;19)(q23;p13) (Figure 12.10) [44]. The biological behavior and clinical course of the disease appear to be similar for both forms. The leukemic cells represent late precursor

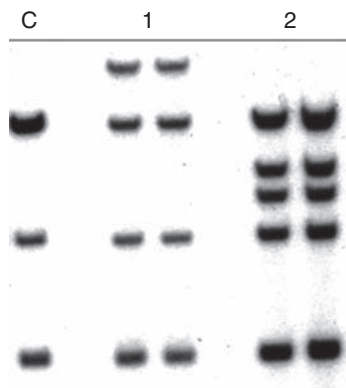


FIGURE 12.4 Southern blot analysis of *BCR-ABL1* translocation in two patients (lanes 1 and 2) with Ph¹-positive adult ALL. Lane C is a negative control sample showing the position of the germline (unrearranged) DNA pattern using this particular restriction endonuclease. Both patients show extra, non-germline hybridizing bands, indicative of a rearrangement caused by the chromosome 9;22 translocation.

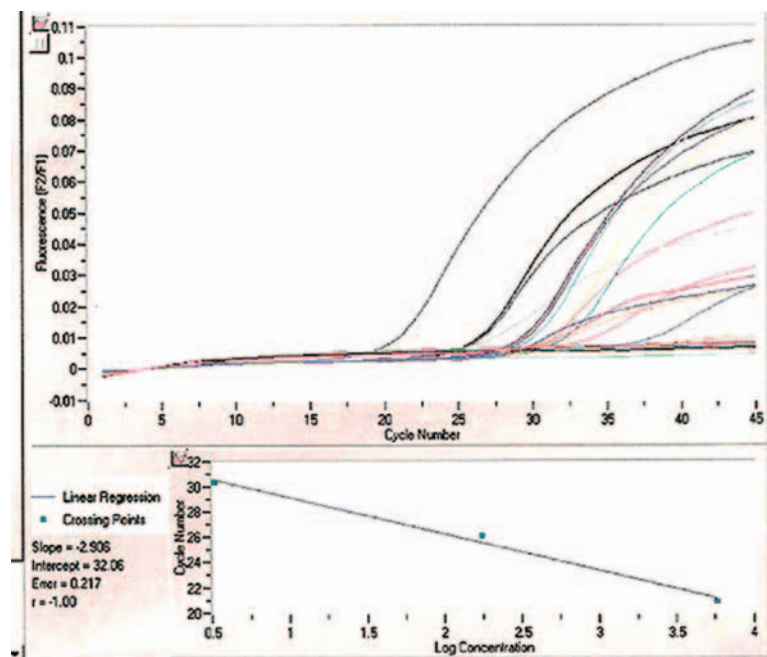


FIGURE 12.5 RT-PCR analysis of a series of patients showing the presence of the *bcr-abl* fusion mRNA target, using the Roche LightCycler instrument. In general, the lower the PCR cycle number (x-axis) at which the amplification reaches its logarithmic phase, the higher the amount of starting *bcr-abl* target sequence in the specimen. Very late-rising and/or low-rising signals should be interpreted with caution.

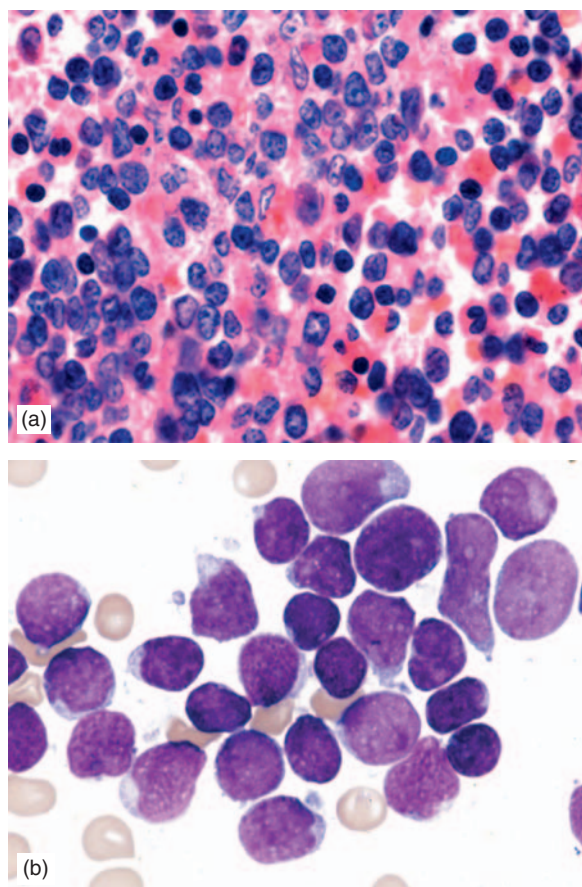


FIGURE 12.6 Bone marrow clot section (a) and smear (b) from a case of acute lymphoblastic leukemia with $t(4;11)$. The blasts are of various sizes and show scanty cytoplasm.

B-cells and are often negative for CD34 and express CD10, CD19, CD20, and cytoplasmic μ [44, 45].

The $t(1;19)(q23;p13)$ leads to a chimeric gene as the result of the fusion of the *PBX1* and *E2A* genes from chromosomes 1 and 19, respectively [43]. The leukemogenic effects of the *PBX1/E2A* fusion protein are not well understood.

Most clinical studies suggest that ALL patients with $t(1;19)$ have a poor prognosis [22, 41, 42, 46].

$t(12;21)(p13;q22)$

This translocation is one of the most frequent genetic abnormalities in childhood ALL, accounting for about 25% of the cases [22, 47, 48]. It is less frequent in adults, occurring in 3–4% of the patients with B-ALL/B-LBL. The $t(12;21)$ leads to the fusion of the *TEL* (*ETV6*) gene on chromosome 12 with the *AML1* (*RUNX1*) gene on chromosome 21 [22, 47, 48]. It is interesting to know that the persistence of the *TEL-AML1* (*ETV6-RUNX1*) transcript has been reported in some patients with $t(12;21)$ ALL in clinically long-term remission [49].

Because of the similarity of the size and banding patterns of 12p and 21q, the $t(12;21)$ in routine karyotyping is cryptic and its detection may be difficult. Therefore, RT-PCR and FISH are recommended techniques for the detection of this translocation (Figure 12.11) [50]. The dual-color dual-fusion FISH probes in addition to finding the expected 12;21 translocation can also detect extra *RUNX1* signals without the *ETV6-RUNX1* fusion, indicative of the existence of cells with a hyperdiploid karyotype or gene amplification. It is important to recognize the distinction between polysomy 21 (often seen in high hyperdiploidy with good prognosis) (Figure 12.12) and *RUNX1* amplification (associated with poor prognosis) (Figure 12.13). In the

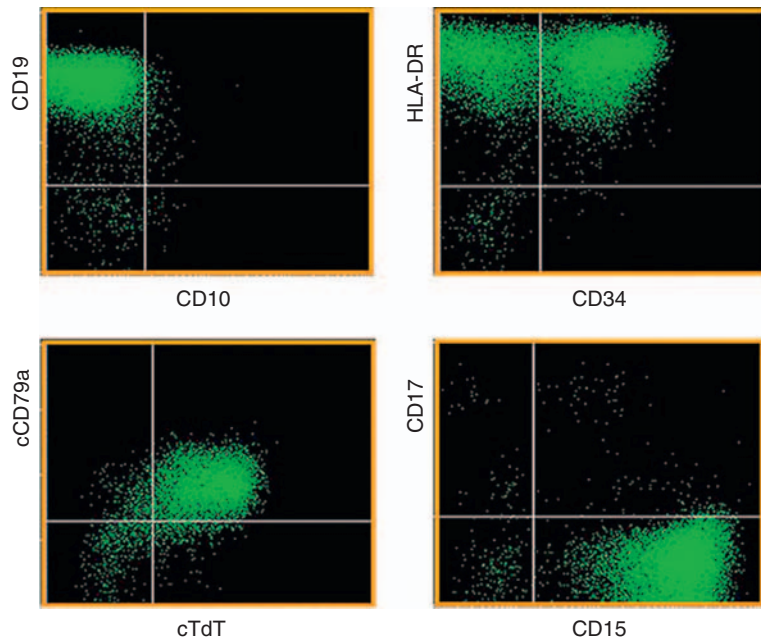


FIGURE 12.7 Flow cytometric analysis of bone marrow from a patient with t(4;11) acute lymphoblastic leukemia. The blast cells are CD10-negative but express CD15, CD19, CD34, HLA-DR, cytoplasmic CD79a and TdT.



FIGURE 12.8 G-banded karyotype of bone marrow of a patient with acute lymphoblastic leukemia demonstrating t(4;11)(q21;q23) (arrows).

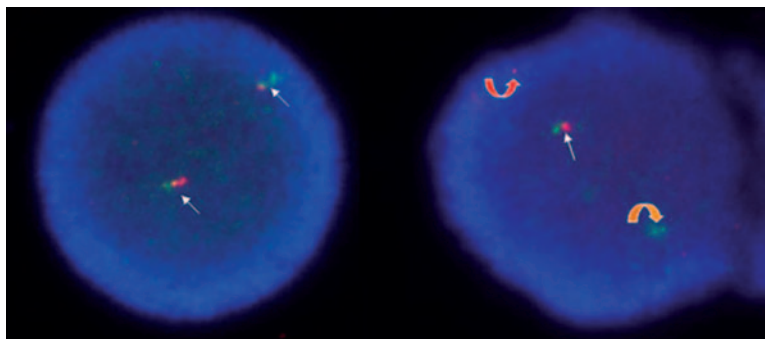


FIGURE 12.9 FISH analysis of bone marrow of a patient with acute lymphoblastic leukemia. The arrows on the left image show normal control and the curved arrows on the right image demonstrate t(4;11).

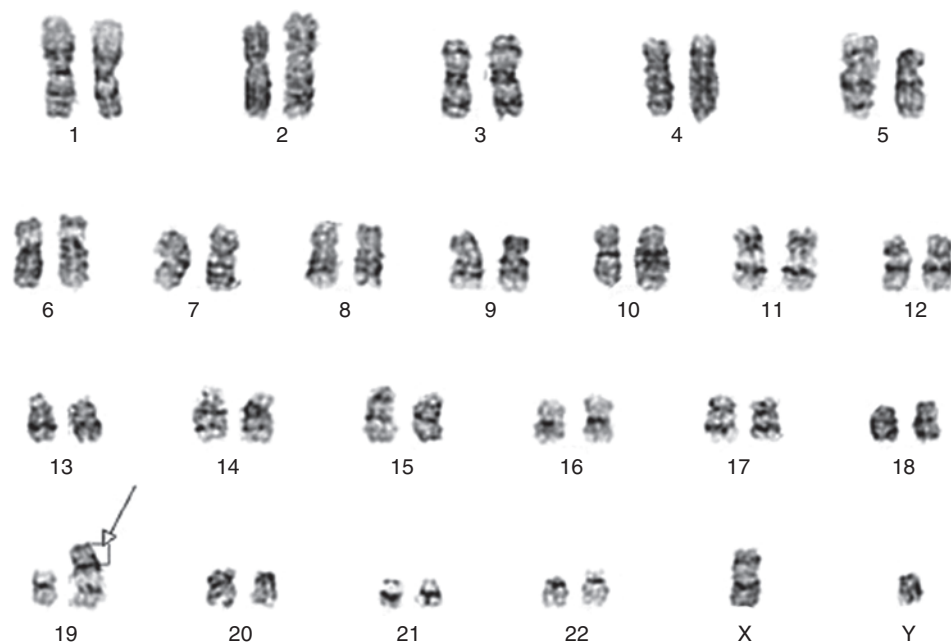


FIGURE 12.10 G-banded karyotype of bone marrow of a patient with acute lymphoblastic leukemia demonstrating der(19)(1;19)(q23;p13.3) (arrow).

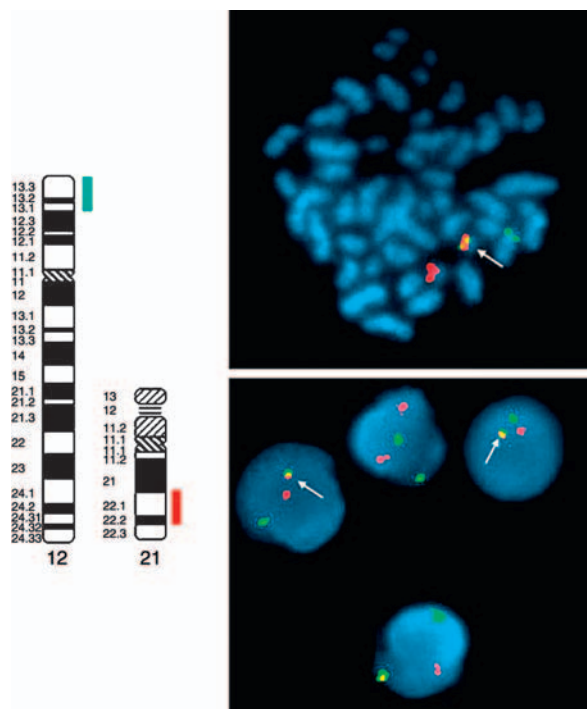


FIGURE 12.11 FISH analysis of bone marrow of a patient with acute lymphoblastic leukemia demonstrating t(12;21)(p13;q22) (arrows).

latter the gene signals are clustered, numbering greater than five or more copies.

The leukemic patients with t(12;21) have a favorable prognosis. They have a much higher 5-year event-free survival than those without this translocation (in one report 91% versus 65%, respectively) [22, 47, 48].

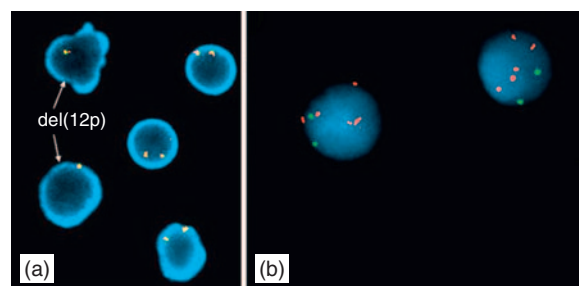


FIGURE 12.12 FISH analysis of bone marrow of a patient with acute lymphoblastic leukemia demonstrating (a) deletion of *ETV6* [del(12p)] and (b) polysomy (five copies) of *RUNX1* (red signals).

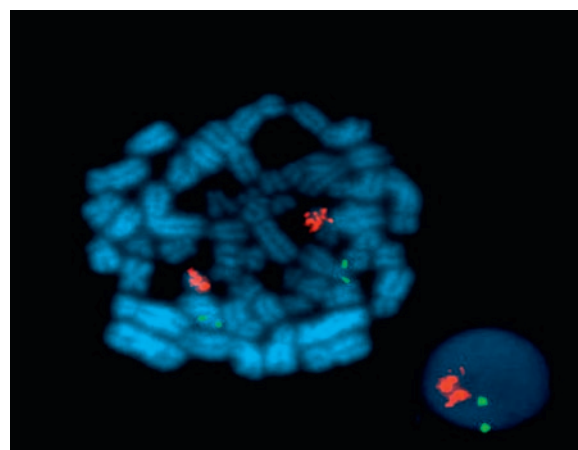


FIGURE 12.13 FISH analysis of bone marrow of a patient with acute lymphoblastic leukemia demonstrating *ETV6* (green signals) and amplification of the *RUNX1* (red signals).

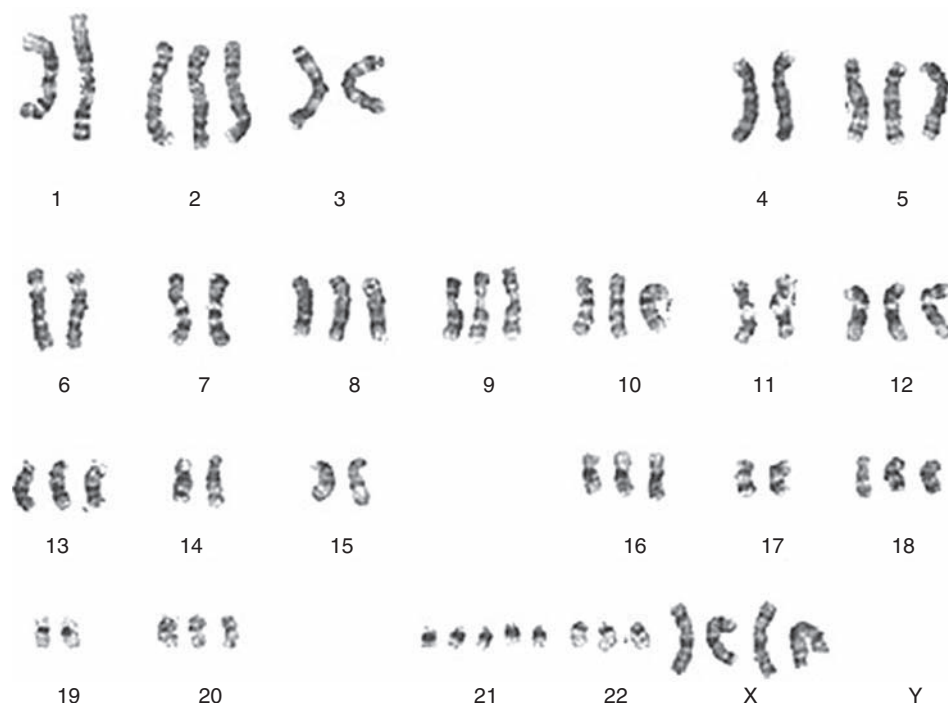


FIGURE 12.14 Hyperdiploid karyotype 62, XX, +X, +X, +2, +5, +8, +9, +10, +12, +13, +16, +18, +20, +21 \times 3, +22.

Hyperdiploidy

Hyperdiploidy is divided into two subcategories: (1) hyperdiploidy with ≤ 50 chromosomes (between 46 and 50 chromosomes) and (2) hyperdiploidy with > 50 (usually between 51 and 65) chromosomes (Figure 12.14). Hyperdiploidy is reported in up to 50% of children with precursor B-ALL [2], but it is far less frequent in adult patients. The most common chromosomal additions include chromosomes 21 (often multiple copies), 4, 6, 10, 14, 17, 18, 20, X, and duplication of 1q and isochromosome 17q [51, 52]. Hyperdiploidy indicates favorable prognosis, particularly in association with trisomy of chromosomes 4, 6, and 10 [2, 51, 52].

Hypodiploidy

This condition refers to having chromosome numbers of < 45 and DNA index of < 1 (Figure 12.15). Several reports indicate poor prognosis in association with hypodiploidy in both children and adult patients with ALL [52–56]. In a large study [56] children with hypodiploid ALL were divided into three major groups: near-haploid (23–29 chromosomes), low hypodiploid (33–39 chromosomes), and high hypodiploid (42–45 chromosomes). Survival analysis showed a poor outcome for the near-haploid and low hypodiploid groups.

Immunoglobulin Gene Rearrangement Clonality

Though not often needed to confirm the diagnosis of these malignancies, precursor B-ALL and LBL should in most cases demonstrate clonal rearrangements of their immunoglobulin genes. Because they are precursor B-cell lesions, they often will not have completed the full ontological sequence of rearrangements, from heavy to light chain. In other words, their transformation to malignancy occurred before their normal maturation could be completed. In such

cases one may see rearrangement of the heavy chain genes (IGH) but not of the light chains (IGK or IGL). Therefore, a greater proportion of these clonal cases will be detected if one uses probes or primers specific for the IGH region. Moreover, some of these cells will not yet have produced antibodies detectable at the protein level, yet the genetic analysis can detect the precursor molecular signature of clonality.

It is important to keep in mind that, unlike the translocations and aneuploidies listed above, immunoglobulin gene rearrangement is a normal process in all B-lymphocytes. It is the fundamental mechanism by which a finite (though diverse) number of genes can be made to encode an almost infinite number of antibody species. By rearranging DNA at these loci (e.g. the heavy chain (IGH) locus on chromosome 14), a specific variable (V) gene (out of the 45 or so available) is brought into contiguity with a specific diversity (D) gene and a joining (J) gene; the VDJ complex is then brought together with a specific constant (C) gene by an RNA splicing event. The structural order of these gene families from 5' to 3' on chromosome 14 is V–D–J–C.

As is the case for *BCR-ABL1* detection, the older Southern blot methods, using DNA probes typically directed at the J-region genes of the IGH region (Figure 12.16), have largely been replaced by PCR approaches. However, as this region is also quite large, it cannot be covered comprehensively by most series of PCR primer pairs, and so some rearrangements in other areas will go undetected (false negatives). Also, antibodies can further diversify themselves through somatic hypermutation of the variable genes, and if any of these changes occur at a primer hybridization site, they can further reduce the efficiency and sensitivity of the assay [57]. For these reasons, many laboratories, including our own, perform an initial screen by PCR, but if that is negative, reflex to the Southern blot procedure. Most PCR approaches detect 70–80% of B-cell neoplasms,

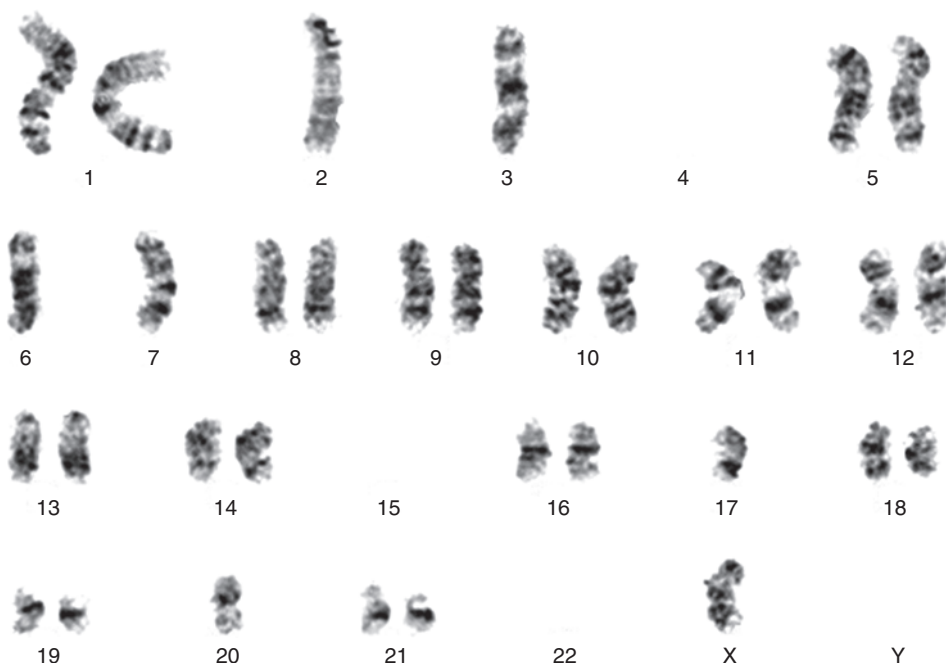


FIGURE 12.15 Hypodiploid karyotype 33, X, -X, -2, -3, -4, -4, -6, -7, -15, -15, -17, -20, -22, -22.

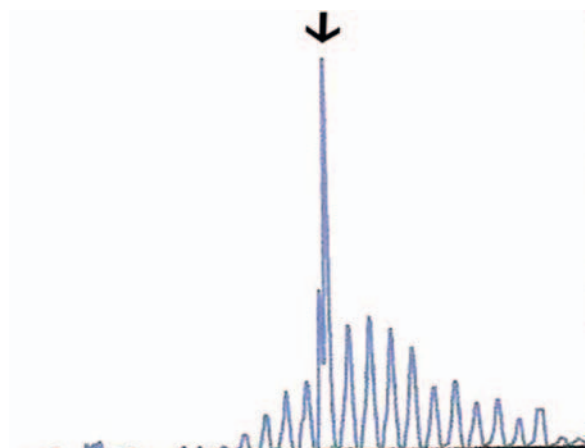


FIGURE 12.16 PCR analysis for immunoglobulin heavy chain clonality. Results are shown for the framework 1 primer set only, illustrating a clonal peak (arrow) superimposed on a polyclonal background population, a pattern often seen in leukemia specimens.

though the sensitivity can be increased somewhat by adding more primer sets for fuller coverage of the region [58]. The most commonly used one is a set of three primer pairs, designated frameworks 1, 2, and 3. Whether done by PCR or Southern blot, the basic principle is the same: a clonal population will generally produce a predominant (or at least visible) DNA pattern (peak or band) above the background of polyclonal population, which should show only germline bands (on Southern blot) or a continuous smear of rearranged bands (on PCR). Germline bands will usually still be seen even in a B-cell malignancy because of the phenomenon of allelic exclusion: the suppression of rearrangement of *IGH* genes on the opposite allele once it has occurred.

In addition, the laboratory will rarely receive a “pure” neoplastic lymphoid specimen; there will virtually always be accompanying benign or non-lymphoid cells in the sample, which will contribute the germline bands. It should also be noted that up to 15% of T-ALLs may rearrange their *IGH* genes, so detection of clonality by this method is not an absolute proof of the lymphocyte subclass of origin.

Clinical Aspects

The incidence of precursor B-ALL in the United States approaches 3 per 100,000 population. The peak incidence is between 2 and 5 years of age, affecting boys more than girls. Acute lymphoblastic leukemia/lymphoma is the most common form of cancer in children, comprising about 30% of all childhood malignancies. Up to 85% of childhood ALLs and 40% of childhood lymphomas are of precursor B-cell type [1, 2, 7, 58]. Lymphoblastic lymphoma is defined by the presence of <25% blasts in the bone marrow and evidence of a mediastinal mass or lymphadenopathy.

Precursor B-LBL is uncommon in adults and accounts for ≤1% of lymphomas. It occurs in younger individuals, usually under 35 years of age. Lymphadenopathy and cutaneous involvement are the most frequent presentations [59]. Skin lesions may be multifocal.

The presenting clinical symptoms are often non-specific and secondary to bone marrow/lymphoid tissue infiltration and pancytopenia, such as fever, bleeding, bone pain, and lymphadenopathy.

Favorable prognostic factors include [1–3, 7]:

- Age younger than 1 or older than 10 years
- White blood cell count in normal range or <50,000/ μ L

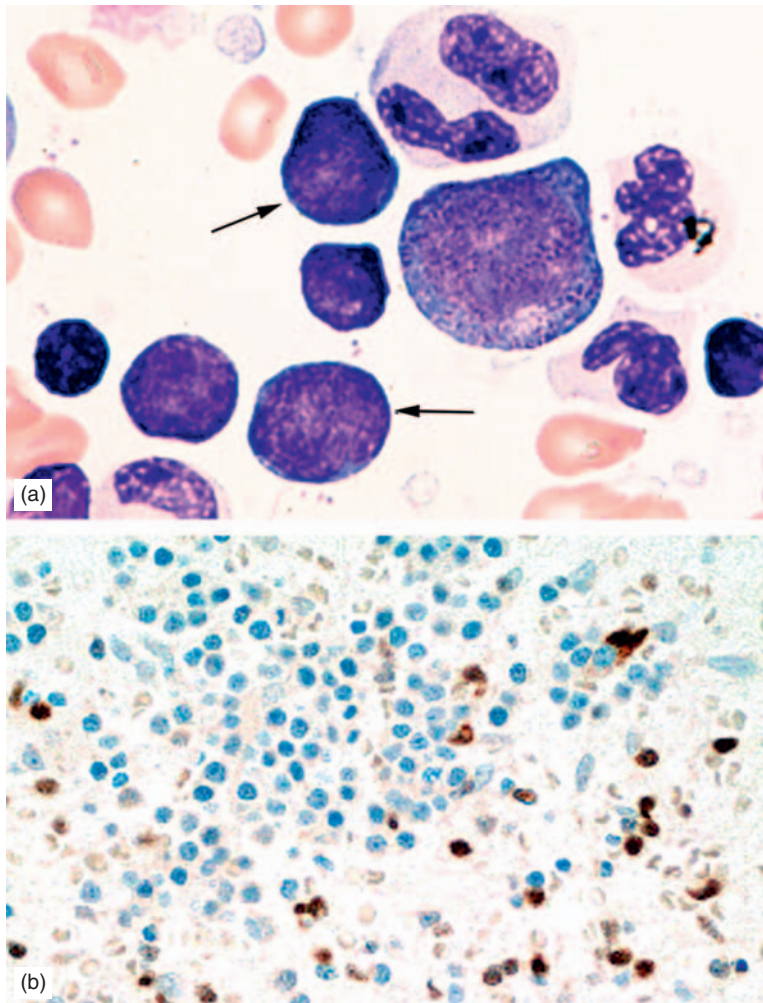


FIGURE 12.17 (a) Bone marrow smear showing scattered hematogones (arrows). (b) Immunohistochemical stain for TdT on a bone marrow biopsy section demonstrating scattered positive cells representing hematogones.

- Hyperdiploidy
- $t(12;21)(p13;q22)$.

The overall response to therapy is significantly better in children than in adults. The current 5-year survival rate for precursor B-ALL in children is approaching 85%.

Differential Diagnosis

The differential diagnosis of precursor B-ALL includes hematogone hyperplasia; precursor T-ALL; various types of acute myeloid leukemia such as minimally differentiated AML, AML without maturation, and megakaryoblastic leukemia; and metastatic small (round) cell tumors such as neuroblastoma.

Hematogones represent the normal bone marrow precursor B-cells. These cells consist of a heterogeneous population. The earlier forms often express TdT, CD34, CD10, and CD19. The more mature forms lose CD34 and TdT and gain CD20 expression. These cells may morphologically resemble lymphoblasts, but usually show somewhat denser nuclear chromatin and absent or inconspicuous nucleoli

(Figure 12.17). Hematogones display a distinctive SSC versus CD45 pattern in flow cytometric studies (Figure 12.18). They are CD45^{dim} and appear as the tail of the mature lymphocytes (CD45^{strong}), together creating a triangular shape. They are found below the blast cells on the SSC/CD45 dot plot. Hematogones account for about 5–10% of the bone marrow cells in children and <5% of the bone marrow cells in adults, but they may be increased in various conditions such as iron deficiency anemia, immune-associated thrombocytopenia, and the following cytotoxic chemotherapy. One of the major difficulties is the distinction between postchemotherapy hematogone hyperplasia and residual disease in children with precursor B-ALL. Both hematogones and residual ALL cells may express CD34, CD10, CD19, and TdT, but they usually have different flow cytometric dot plot patterns. Also, hematogones are scattered through the bone marrow, usually do not form clusters (Figure 12.17b) and are polyclonal, and do not show cytogenetic aberrations; whereas residual precursor B-ALL cells often appear in clusters, are monoclonal, and may show cytogenetic aberrations.

The differential diagnosis of precursor B-LBL includes precursor T-LBL, Burkitt lymphoma, blastic variant

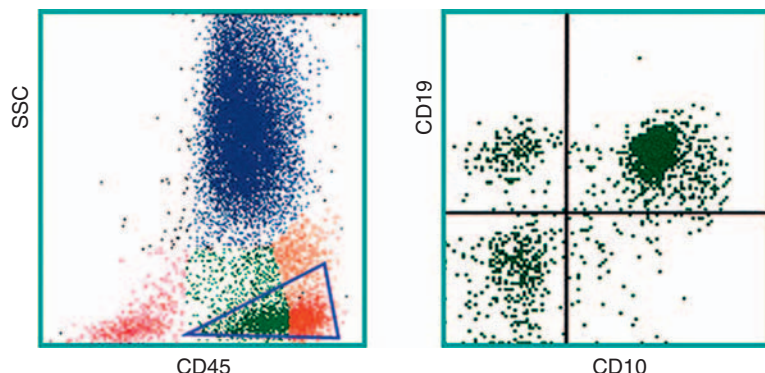


FIGURE 12.18 Flow cytometry of bone marrow hematogones. Hematogones are CD45^{dim} (green population) and usually located on the left side of the lymphocytes (red population) on SSC/CD45 dot plot analysis, as a tail of a triangle. They are B-cells and express CD10.

of mantle cell lymphoma, granulocytic sarcoma, and metastatic small (round cell) tumors. Burkitt and blastic mantle cell lymphomas are TdT negative and have characteristic cytogenetic abnormalities (see Chapter 15).

Precursor B-ALL is distinguished from precursor T-ALL based on their immunophenotypic and cytogenetic characteristics. AML blasts express myeloid-associated markers, such as CD13, CD33, and CD117, and may show positive cytochemical staining for MPO and Sudan Black B.

Metastatic round cell tumors are negative for lymphoid- and myeloid-associated CD molecules and positive for markers that are expressed by the primary tumor.

PRECURSOR T-LYMPHOBLASTIC LEUKEMIA/LYMPHOBLASTIC LYMPHOMA

Precursor T-lymphoblastic leukemia/lymphoma (T-ALL/T-LBL) may initially present itself as ALL with the involvement of bone marrow and/or blood, or LBL with the involvement of the lymphoid and/or other extramedullary tissues [1, 2]. In a significant proportion of cases, however, both bone marrow and extramedullary tissues are involved. T-ALL and T-LBL are regarded as different clinical presentations of the same disease.

Etiology and Pathogenesis

The etiology and pathogenesis of precursor T-ALL and T-LBL are not known. Epidemiological studies, as briefly discussed earlier, point to chromosomal instability and exposure to ionizing radiation, certain chemicals, viruses, or bacteria as risk factors for the development of T-ALL.

The most frequent chromosomal translocations in precursor T-ALL result in the activation of proto-oncogenes such as *TAL1-STIL*, *LYL1*, *LMO1*, *LMO2*, *TAN*, and *MYC*, usually by positioning these genes next to the regulatory elements of the T-cell receptor (TCR) gene [24, 60, 61]. These genes are considered to be transcription regulators

and their inappropriate expression may play a role in leukemogenesis [62].

Pathology

Morphology

The morphologic features of precursor T-ALL and T-LBL are similar to those of precursor B-ALL and B-LBL. The bone marrow biopsy and clot sections usually are hypercellular and diffusely infiltrated by sheets of uniformly appearing blast cells (Figure 12.19). These cells have scanty cytoplasm with round, oval, or indented nuclei; finely dispersed nuclear chromatin; and prominent or indistinct nucleoli. In some cases, the leukemic blast cells may appear pleomorphic with variable amounts of cytoplasm or may show convoluted nuclei. Mitotic figures are more frequently observed in T-ALL than in B-ALL [1, 2]. Bone marrow fibrosis and osteoporosis are sometimes present. Fibrosis may be mild, extensive, focal, or diffuse, and it may lead to unsuccessful bone marrow aspiration (dry tap). It is more frequent in B-ALL than in T-ALL. Large areas of bone marrow necrosis are infrequent. Necrosis is usually of the coagulative type with the preservation of the basic outline of the necrotic cells.

Similar to the precursor B-ALL, bone marrow smears and touch preparations show numerous blast cells that are often small with scanty non-granular blue cytoplasm, fine chromatin pattern, and indistinct nuclei. In a minority of the cases, the blast cells are pleomorphic and may show azurophilic granules.

Lymphoblasts may also be present in the peripheral blood smears in variable numbers. They account for the majority of leukocytes in patients with WBC >10,000/ μ L. Anemia, granulocytopenia, and/or thrombocytopenia are common features.

The involvement of lymph nodes and other tissues is usually diffuse with total or partial effacement of the normal architecture and morphologic features similar to those described earlier in the bone marrow biopsy sections. In some cases, the high rate of tumor cell turnover and necrosis may stimulate the macrophages. These macrophages with abundant pale, vacuolated cytoplasm and phagocytic cell debris are dispersed throughout the lymphomatous lesion, creating a “starry sky” pattern.

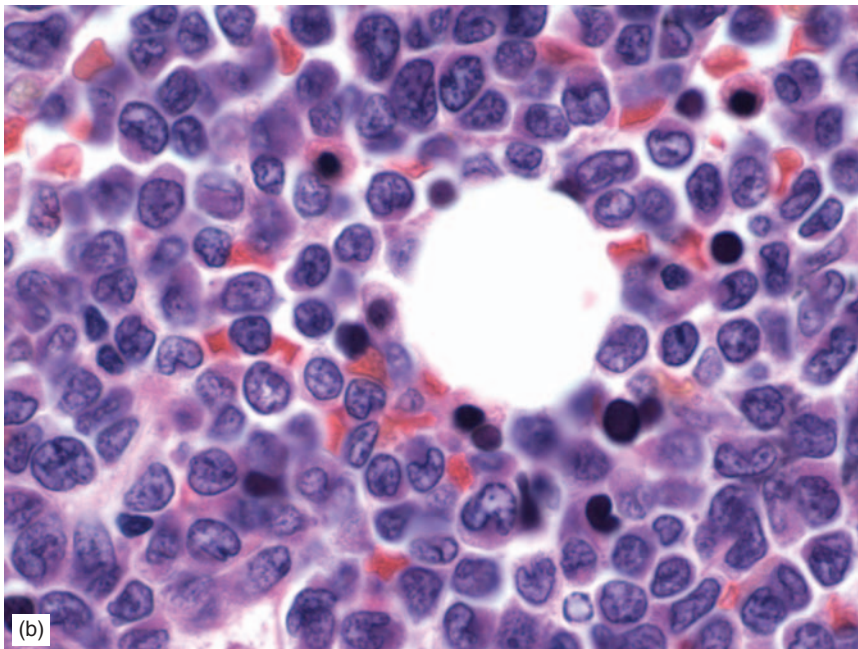
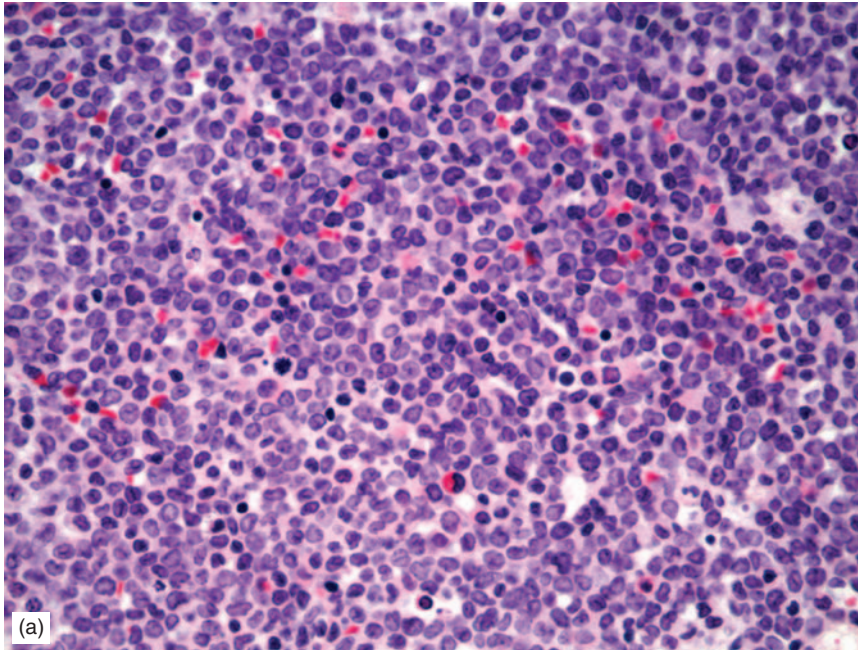


FIGURE 12.19 Bone marrow biopsy section from a patient with precursor T-acute lymphoblastic leukemia showing sheets of blast cells with irregular nuclei (a) low power and (b) high power.

Immunophenotype and Cytochemical Stains

The vast majority of precursor T-ALL/T-LBL cases characteristically express nuclear TdT, cytoplasmic CD3, and CD7 (Figures 12.19b and 12.20). The neoplastic T-blast cells are frequently CD34-positive and express weak CD45 [2, 63–67]. The precursor T-cells are divided into three phenotypic subcategories (Table 12.3):

- Early T-cell
- Intermediate thymocyte
- Late thymocyte.

The early precursor T-blast cells, in addition to TdT, CD7, and cytoplasmic CD3, express CD2 and CD38. The intermediate precursor cells characteristically express CD1a, surface CD3, CD4, and CD8, and the blasts of the late stage thymocyte category express CD4 or CD8 (Table 12.3). The intermediate precursor T-ALL/T-LBL type is the most frequent one (common type), accounting for over 50% of the cases. In general, cases of T-LBL may represent more of mature T-cell phenotype than those of T-ALL.

Approximately half of the T-LBL cases also express CD44 (homing receptor/cell adhesion molecule) [2]. Aberrant expression of myeloid-associated markers, such

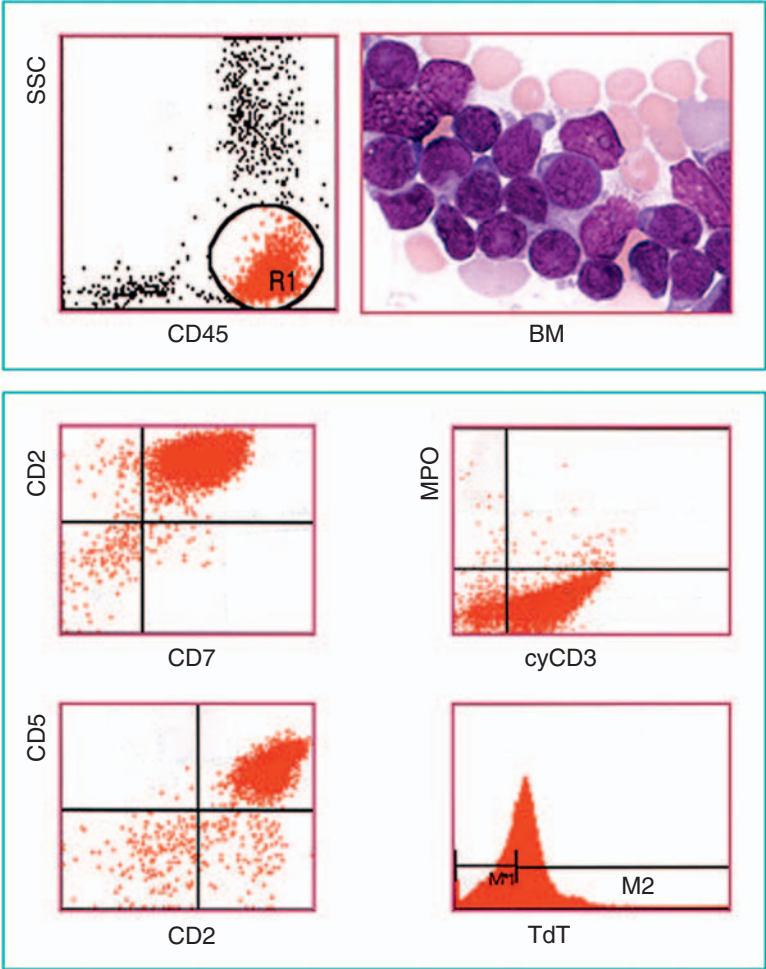


FIGURE 12.20 Flow cytometric analysis of bone marrow from a patient with precursor T-lymphoblastic leukemia demonstrating a population of CD45+ blast cells also expressing CD2, CD5, CD7, and cytoplasmic CD3 (cyCD3), and TdT.

TABLE 12.3 Immunophenotypic characteristics of T-cells in various stages of maturation.

Stage	Immunophenotype
Early precursor	CD7, TdT, cCD3
Intermediate precursor (common thymocyte)	CD7, TdT, cCD3, CD1a, CD2, CD5, CD38, variable CD4, variable CD8
Late stage (late thymocyte)	CD7, CD5, CD3, CD2, CD45, CD4 or CD8

as CD13 and CD33, is not infrequent [67, 68]. Occasional cases may also express CD117 or CD79a. A significant proportion of cases of T-ALL and T-LBL express CD10, whereas CD16 or CD56 expression is infrequent [58].

No lineage-specific cytochemical stains are available for precursor T-blast cells. These cells may show focal acid phosphatase or NSE reactions.

Cytogenetic and Molecular Studies

Various genes serve as partners in these translocations, such as *C-MYC*, *TAL1/SCL*, *LMO1(RBTN1)*, and *HOX11*, located on chromosomes 8q24, 1p32, 11p15, and 10q24, respectively [2, 24, 69]. Precursor T-cell neoplasms may show evidence of either or both *TCR* and *IGH* gene rearrangements [60, 61].

Quantitative chromosomal abnormalities include del(6q) (Figure 12.21), del(9p) (Figure 12.22), and trisomy 8 (Table 12.4). Approximately 30% of cases of T-ALL/T-LBL show translocations involving 14q11.2 (*TCRα/δ*) or 7q34 (*TCRβ/γ*) (Table 12.4), such as t(11;14)(p15;q11.2), (14;21)(q11.2;q22), and t(7;14)(q34;q11.2) (Figure 12.23) [22, 62, 70]. Inversion of chromosome 6 [inv(6)(p21.2q27)] has been reported in association with precursor T-ALL [71–73].

Generation of TCR molecules in T-cells occurs by much the same mechanism as the generation of immunoglobulin molecules in B-cells. Therefore, clonality of T-cell malignancies can be demonstrated by examining the rearrangement patterns of the TCR genes, in much the same way as is done with the immunoglobulin genes in the B-cell lesions. *TCR* rearrangements are somewhat more complicated, however, and there are advantages and disadvantages to the various target loci available. For example, the *TCR-β* genes produce a wider range of rearrangements and thus are more informative, but the region is so large that a high number of PCR primer sets are required to span it. The *TCR-γ* region, in contrast, is smaller and easily encompassed by a small number of primer sets, but the limited number of potential rearrangements increases the chance of the artifact known as pseudoclonality, in which amplification of a specimen in which T-cells are scanty will produce

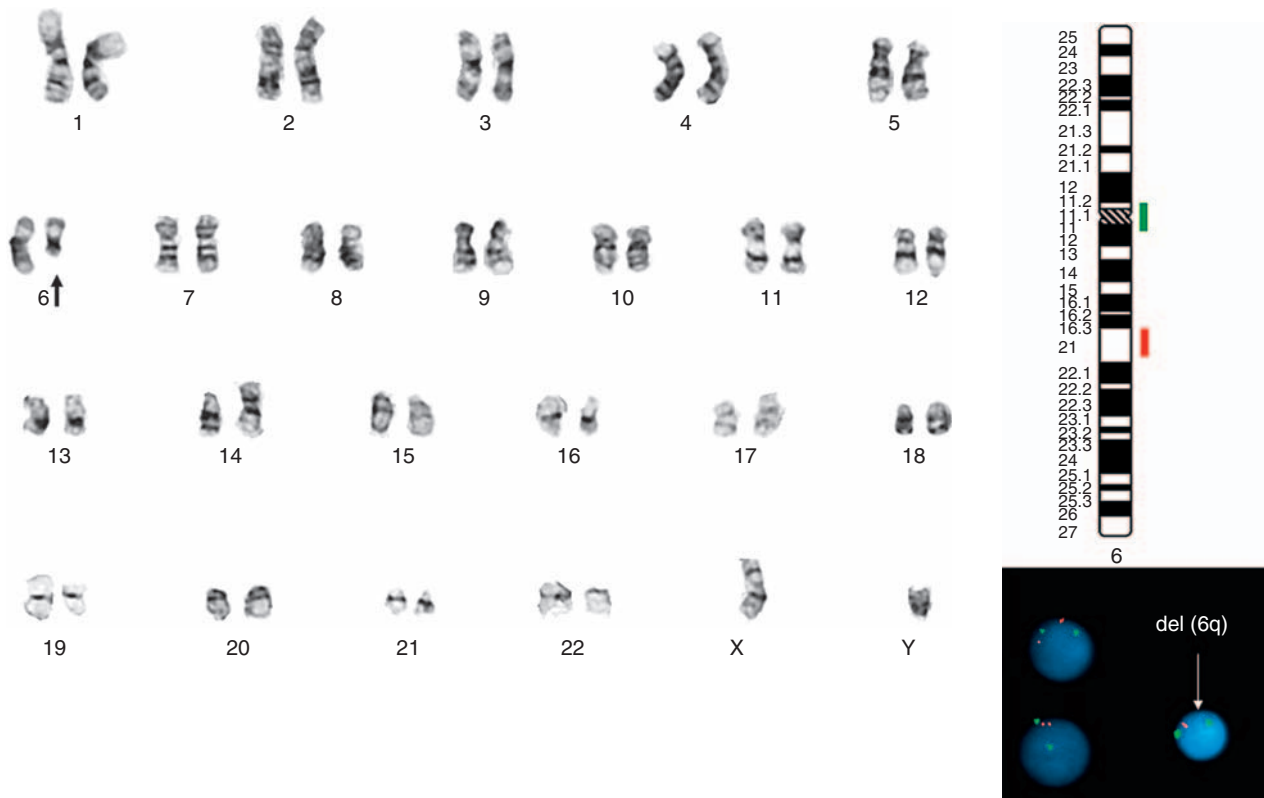


FIGURE 12.21 G-banded karyotype and FISH analysis demonstrating 46,XY,del(6)(q15).



FIGURE 12.22 G-banded karyotype demonstrating 46,XY,del(9)(p21).

an apparent clonal rearrangement pattern even though the cells are neither malignant nor clonal. Still, TCR- γ PCR is most often used by virtue of its practicality [74] and because these genes customarily rearrange before the TCR- β genes do, but a negative result will often require reflex to Southern

blot analysis in these disorders, at least for those laboratories that choose not to do the extensive TCR- β PCR coverage. On the plus side, TCR genes do not undergo somatic hypermutation as do the immunoglobulin genes, and so is not a source of false negatives. Molecular analysis is more

often required in the precursor T-cell lesions because of the absence of surface molecules detectable by immunologic methods and flow cytometry. However, as is the case for the B-cell lesions, detection of *TCR* gene rearrangements does not absolutely prove T-cell origin, since a minority of B-ALLs will also rearrange these genes.

TABLE 12.4 Recurrent cytogenetic abnormalities in precursor T-lymphoblastic neoplasms.

Chromosomal aberrations	Affected genes
t(1;7)(p32;q35)	<i>TAL1/TCRβ</i>
t(1;14)(p32;q11.2)	<i>TAL1/TCR$\alpha\delta$</i>
T(7;10)(q35;q24)	<i>TCRβ/TAL3*</i>
t(7;19)(q35;p13)	<i>TCRβ/TAL2**</i>
t(8;14)(q24;q11.2)	<i>MYC/TCR$\alpha\delta$</i>
t(11;14)(p15;q11.2)	<i>TTG1/TCR$\alpha\delta$</i>
t(11;14)(p13;q11.2)	<i>TTG2/TCR$\alpha\delta$</i>
inv(14)(q11.2;q32)	<i>TRC$\alpha\delta$/TCL1</i>
del(1p32)	<i>TAL1</i>
del(6q)	
del(9p)	
+8	

Alternative designations:
**HOX11*, *SCL* or *TCL5*.
***TAN*.

It should also be noted that the gene rearrangement clonality studies as described here are less suitable for detecting minimal residual disease than the PCR-based methods that target translocations such as *BCR-ABL1* and the others listed in this chapter. The reason is that the translocations are not found in normal cells and non-pathologic states, so the PCR primers theoretically have no competition from normal targets that would lower the overall sensitivity of the assay. In contrast, *IGH* and *TCR* gene rearrangements are normal phenomena in all lymphocytes, and the same primer hybridization sites are present, with the same efficiency in both benign and neoplastic lymphocytes. This lowers the sensitivity of the detection of the malignant clone to a level of only 5–10%, compared to 1 in 100,000 or less for the PCR-based translocation assays.

A more recent technique, gene expression or mutation profiling by microarray hybridization, may provide valuable information regarding the biology of these disorders and their response to therapy (Figure 12.24) [75].

Clinical Aspects

Precursor T-cell neoplasms represent about 15% of ALLs in children and 25% in adults. Approximately 2% of adult non-Hodgkin lymphomas are precursor T-cell type [2, 7, 76, 77]. Most of the patients are adolescent or young adults with male preponderance, and the vast majority of the patients are at stage III or IV at the time of diagnosis. Anterior mediastinal mass and/or peripheral lymphadenopathy is detected in between 50% and 75% of the cases. Cervical, supraclavicular, and axillary lymph nodes are frequent targets. Extranodal tissues such as skin, testicle, or bone are involved less frequently. A high frequency of CNS involvement has been noted in patients with T-ALL. The overall prognosis of the precursor T-cell neoplasms is worse

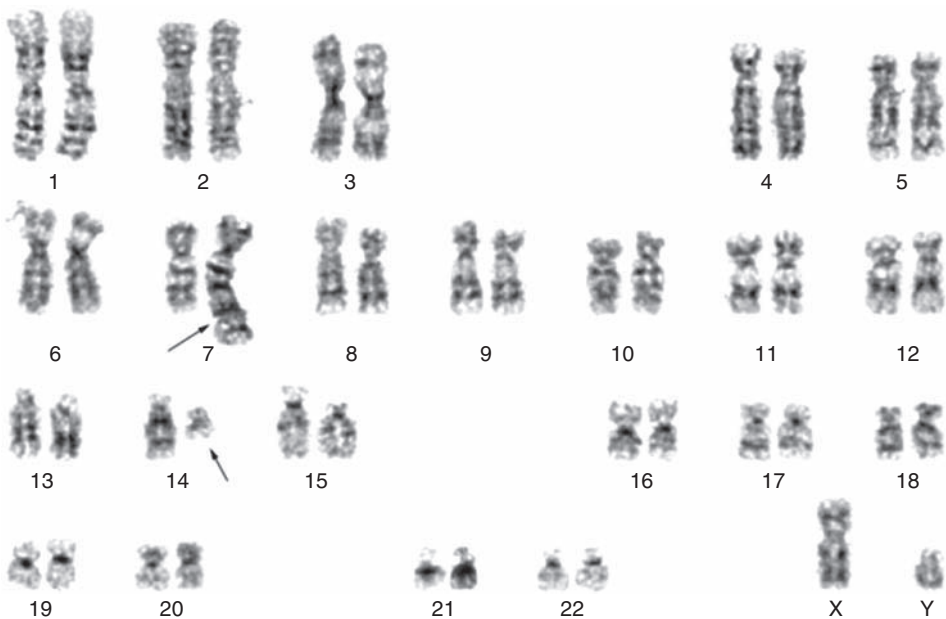


FIGURE 12.23 G-banded karyotype of bone marrow of a patient with acute precursor T-lymphoblastic leukemia demonstrating t(7;14) (q35;q11.2) (arrows).

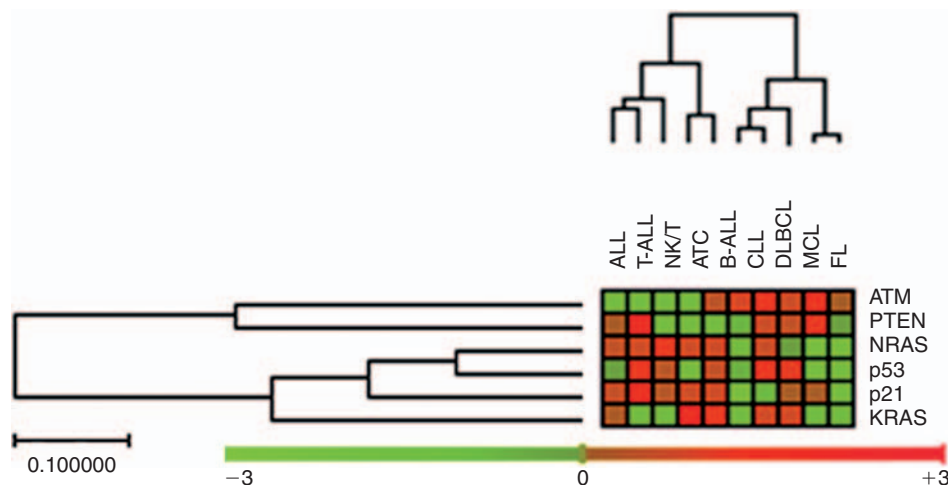


FIGURE 12.24 Schematic correlation of gene mutation profiles in acute lymphoblastic leukemias (ALL) and certain types of lymphomas. Data used from www.sanger.ac.uk/genetics/CGP/cosmic. Courtesy of Dr. Dejun Shen. T-ALL: precursor T-lymphoblastic leukemia/lymphoma, NK/T: NK/T-cell type lymphoma, ATC: adult T-cell leukemia/lymphoma, B-ALL: precursor B-acute lymphoblastic leukemia/lymphoma, DLBCL: diffuse large B-cell lymphoma, MCL: mantle cell lymphoma, and FL: follicular lymphoma.

than that of their B-cell counterparts. So far, no clear-cut correlation has been found between the prognosis and the immunophenotypic or cytogenetic results. Consolidation with high-dose therapy and autologous or allogeneic stem cell transplantation are considered in young patients [76,77].

Differential Diagnosis

The differential diagnosis of precursor T-cell neoplasms includes hematogone hyperplasia; precursor B-ALL/B-LBL; various acute myeloid leukemias such as minimally differentiated AML, AML without maturation, and megakaryoblastic leukemia; and metastatic small (round) cell tumors such as neuroblastoma.

Hematogones represent the normal bone marrow precursor B-cells. The earlier hematogones may express TdT, but they coexpress TdT and lack the expression of T-cell markers. As was mentioned earlier, hematogones display a distinctive SSC versus CD45 pattern in flow cytometric studies (see Figure 12.15). Hematogones account for about 5–10% of the bone marrow cells in children and <5% of the bone marrow cells in adults. Hematogones are evenly distributed in the bone marrow interstitium, are polyclonal, and do not show cytogenetic aberrations.

The differential diagnosis of precursor T-LBL includes precursor B-LBL, Burkitt lymphoma, blastic variant of mantle cell lymphoma, granulocytic sarcoma, and metastatic small (round cell) tumors. Burkitt and blastic mantle cell lymphomas are TdT negative, express B-cell-associated CD molecules, and have characteristic cytogenetic abnormalities (see Chapter 15).

Precursor T-ALL is distinguished from precursor B-ALL based on their immunophenotypic and cytogenetic characteristics. AML blasts express myeloid-associated markers, such as CD13, CD33, and CD117; lack cytoplasmic CD3 expression; and may show positive cytochemical staining for MPO and Sudan Black B.

Metastatic round cell tumors are negative for lymphoid- and myeloid-associated CD molecules and positive for markers that are expressed by the primary tumor.

OTHER LYMPHOBLASTIC LEUKEMIA/LYMPHOMA VARIANTS

NK-Cell Lymphoblastic Leukemia/Lymphoma

The blast cells in a small proportion of ALL/LBL express CD56 and lack B-, T-, and myeloid-associated markers, do not show Ig and *TCR* gene rearrangements and are negative for EBV [79–81]. They are CD45^{dim} and may express CD2, CD7, TdT, and/or CD16, and often lack cytoplasmic azurophilic granules. These tumors are clinically aggressive and have a poor prognosis.

ALL with Cytoplasmic Granules

In a small proportion of patients with ALL (4–7%), particularly in children, lymphoblasts contain coarse azurophilic granules [82–86]. These granules are usually larger than the granules in myeloblasts and are MPO-negative by conventional light microscopic examination. However, they may display MPO positivity by electron microscopy. ALL with cytoplasmic granules is usually of precursor B-cell type.

ALL with Eosinophilia

Eosinophilia has been reported in rare cases of ALL [87–89]. These patients may show clinical symptoms of hypereosinophilic syndrome. ALL with eosinophilia is often

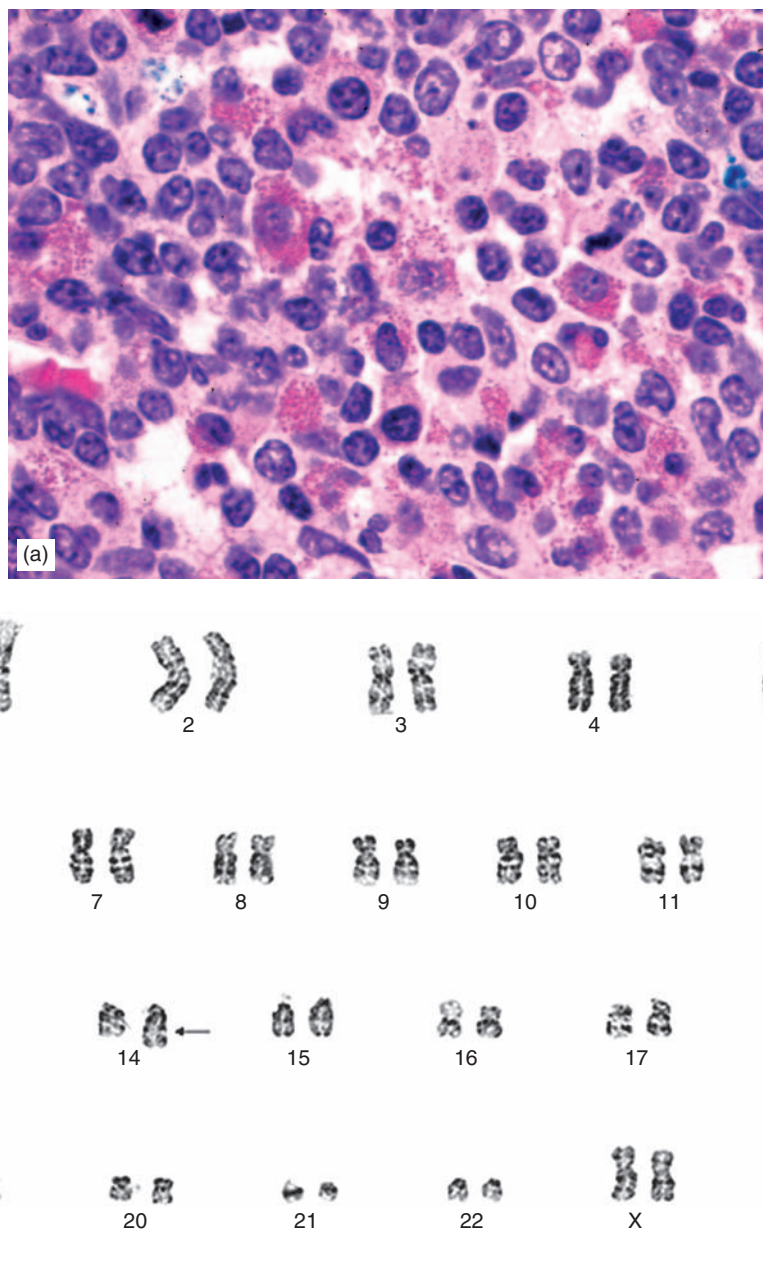


FIGURE 12.25 (a) Bone marrow biopsy section of a patient with precursor B-acute lymphoblastic leukemia and eosinophilia. (b) G-banded karyotyping showing t(5;14)(q31;q32).

of the precursor B-cell type and has been reported frequently in association with t(5;14)(q31;q32), involving *IL-3* (5q31), and *IGH* (14q32) genes (Figure 12.25). Increased expression of IL-3 is thought to be responsible for the marked eosinophilia. ALL with eosinophilia has an aggressive clinical course.

ALL Preceded by or Associated with Hypoplastic Marrow

Occasionally ALL patients may initially present with a hypoplastic marrow and pancytopenia mimicking aplastic anemia [90–92]. The hypoplastic marrow contains a variable

number of lymphoblasts (Figure 12.26) and may eventually become packed with lymphoblasts with an obvious acute leukemia picture. This condition, unlike hypoplastic AML, is not usually associated with myelodysplastic changes and is often of precursor B-cell type.

ALL, Burkitt Type

Acute leukemia of Burkitt type in the WHO classification is considered a clinical spectrum (leukemic phase) of Burkitt lymphoma. The neoplastic cells are monomorphic medium-sized lymphoid cells with scanty deep blue and vacuolated cytoplasm and round and multiple nuclei. These

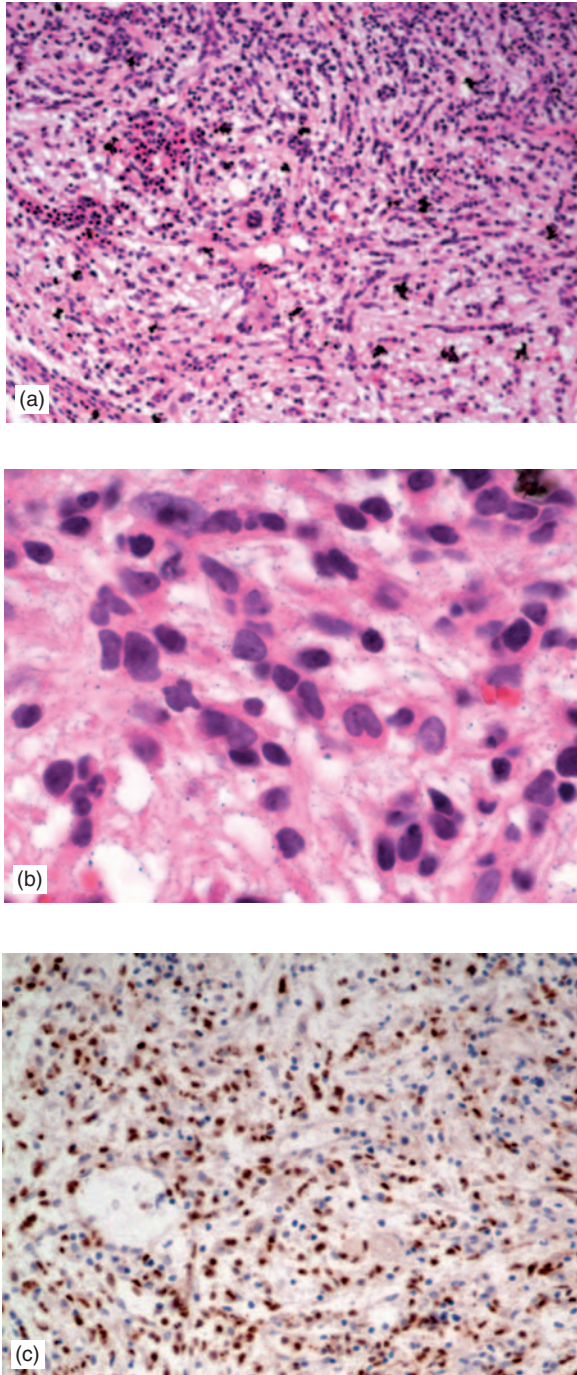


FIGURE 12.26 Bone marrow biopsy section from a patient with hypoplastic acute lymphoblastic leukemia (a) low power and (b) high power. Immunohistochemical stain showing numerous TdT positive cells (c).

cells express surface Ig and lack TdT and CD34 expression. Burkitt leukemia/lymphoma is discussed in Chapter 15 as a subcategory of mature B-cell neoplasms.

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Acute Leukemias of Ambiguous Lineage

The addition of cytochemical and immunophenotypic techniques to the standard morphologic evaluation of leukemic blast cells has led to the growing recognition of acute leukemias with ambiguous lineage assignment [1, 2]. According to the WHO classification, these leukemias fall into two major categories [1]:

1. Acute leukemias which lack sufficient evidence of morphologic, cytochemical, and immunophenotypic features of lineage differentiation (acute undifferentiated leukemia).
2. Acute leukemias which have morphologic and/or immunophenotypic characteristics of both myeloid and lymphoid lineages, or both T- and B-lymphoid cells (acute bilineal and acute biphenotypic leukemias) (Figure 13.1).

ETIOLOGY AND PATHOGENESIS

The etiology and pathogenesis of this heterogeneous group of leukemias are not known. However, recent reports suggest that the mixed-lineage leukemia (*MLL*) gene may play an important role in the development of acute leukemias of ambiguous lineage. For example, studies by Ono and associates suggest that *MLL* fusion products are essential to immortalize hematopoietic progenitors [3]. These investigators demonstrated that *MLL*-SEPT6 fusion protein with activated FMS-like tyrosine kinase 3 (*FLT3*) together could induce acute biphenotypic leukemia in mice. The *MLL* gene (also called *ALL1* or *HRX*) has been mapped on chromosome 11 (11q23). More than 30 partner genes have been identified in association with 11q23 translocations such as t(4;11), t(9;11), and t(11;19) [3, 4]. Fusion of

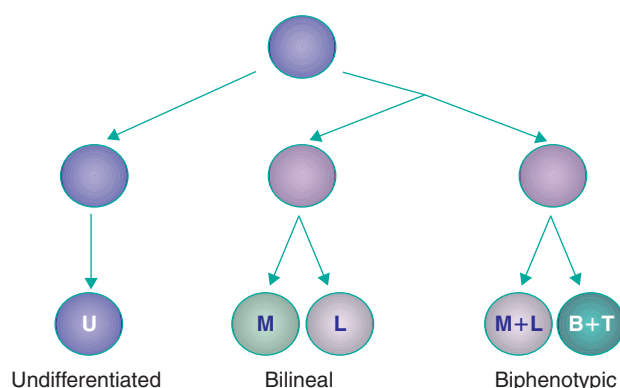


FIGURE 13.1 Scheme of clonal development of acute leukemias of undifferentiated (U), bilineal and biphenotypic types.

MLL with a translocation partner leads to the development of leukemias in experimental animals [3].

PATHOLOGY

Morphology

Morphologic features of acute leukemias of ambiguous lineage are variable. The ones that fall into the category of acute

undifferentiated leukemia show no morphologic features of lymphoid or myeloid differentiation. Blasts consist of primitive undifferentiated cells with no cytoplasmic granules or lineage-associated cytochemical and immunophenotypic features. The bilineal leukemias consist of two distinct populations of blast cells representing both lymphoid and myeloid lineages (Figure 13.2). Lymphoblasts are usually smaller with scanty cytoplasm, no cytoplasmic granules, and less prominent nucleoli; and blasts of myeloid origin (myeloblasts and monoblasts) are larger with more abundant cytoplasm, variable amounts of cytoplasmic granules, and prominent nucleoli.

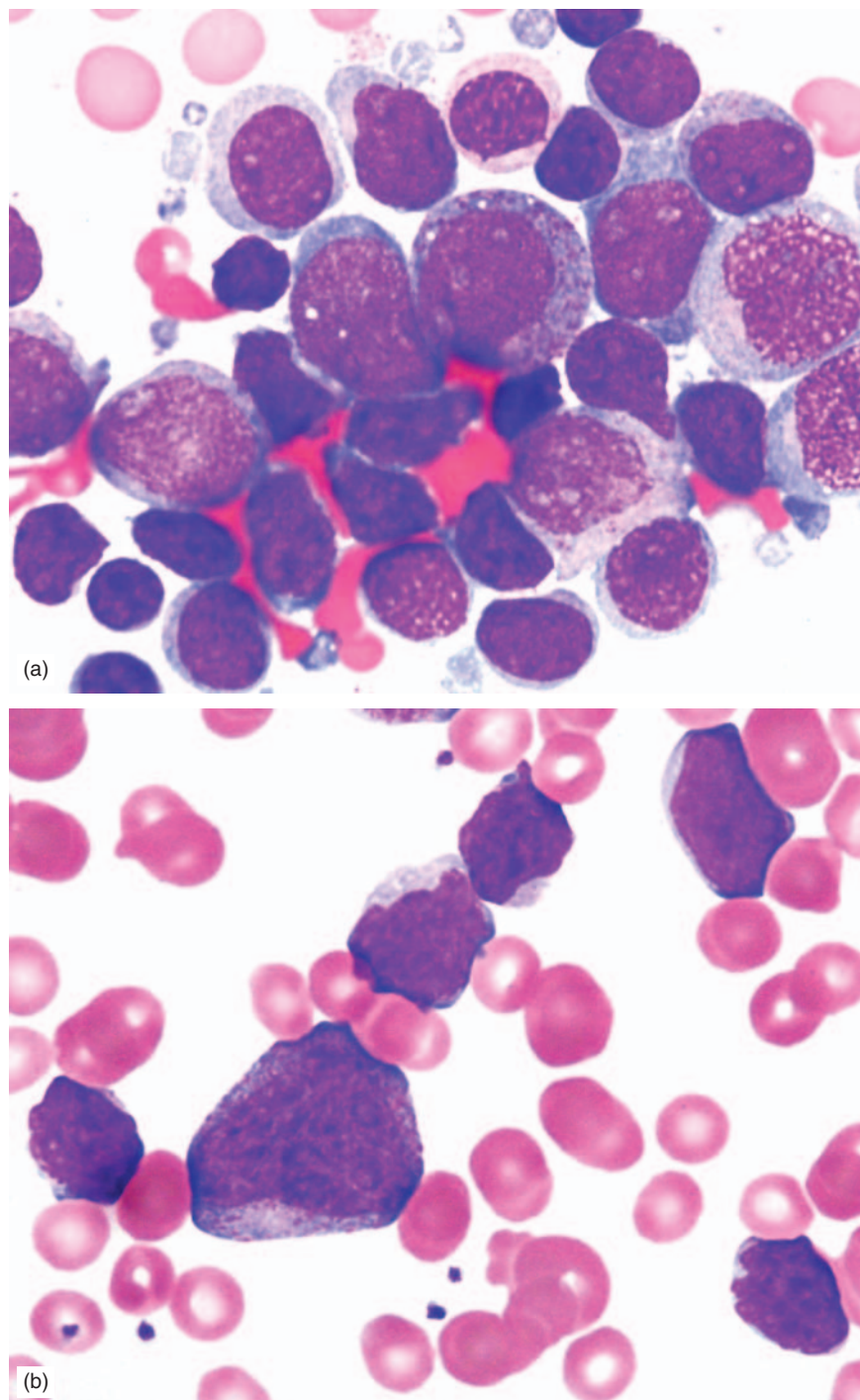


FIGURE 13.2 Bone marrow (a) and peripheral blood (b) smears of a patient with bilineal leukemia. Two distinct populations of leukemic cells (larger and smaller) are present.

These two populations demonstrate distinct cytochemical and immunophenotypic properties (see later).

Immunophenotype and Cytochemical Stains

The primary basis for the characterization of acute leukemias with ambiguous lineage is their immunophenotypic features. Although the term *biphenotypic* leukemia has been used widely in the literature, the general consensus is that this term should be used more restrictively and only when the leukemic blast cells co-express several markers of both myeloid and lymphoid or B- and T-cells. A scoring system has been proposed by the European Group for the Immunologic Classification of Leukemia (EGIL) for the lineage assignments of leukemias [5] (Table 13.1). The most lineage-specific markers are considered to be cytoplasmic CD22, cytoplasmic CD79a, and cytoplasmic μ for B-cells; CD3 and TCR for T-cells; and MPO for myeloid cells; each receiving a score of 2. The least lineage-specific markers are considered to be TdT and CD24 for B-cells; TdT, CD7, and CD1a for T-cells; and CD14, CD15, and CD64 for myeloid cells; each receiving a score of 0.5. In this scoring system, leukemia is considered biphenotypic when the total scores of the CD markers co-expressed by the leukemic cells are >2 in more than one lineage-associated category. For example, if the same blast cell population in a patient with acute leukemia co-expresses CD79a and CD19 (B-cell markers with total score of 3) and myeloperoxidase and CD15 (myeloid markers with total score of 2.5), the leukemia is considered biphenotypic.

In spite of the recommendation of the WHO for the scoring system proposed by EGIL, there are still some ambiguities regarding the interpretation of phenotypic results for

acute leukemias with ambiguous lineage. For example, the required minimal percentage of positive cells co-expressing myeloid and lymphoid markers has not been defined, nor has the required intensity of the expression of myeloid- and lymphoid-associated markers been clarified.

The vast majority of the acute leukemias with aberrant expression of lymphoid or myeloid markers are not qualified to be called biphenotypic or bilineal. They are mostly considered to be AMLs with aberrant expression of lymphoid markers or ALLs with aberrant expression of myeloid markers (Table 13.2).

Acute Leukemias with Expression of Aberrant Markers

This category consists of:

- A. Acute myeloid leukemias with aberrant expression of lymphoid markers (AML + L) [6–10]. In this type of leukemia the total immunophenotypic score for the myeloid markers is >2 whereas that for lymphoid-associated markers is ≤ 2 (Figure 13.3). In our experience the most frequent aberrant lymphoid markers expressed by AML cells are CD7, CD19, and CD56. Other frequently reported lymphoid markers in AMLs include CD2, CD22, and CD79a.
- B. Acute lymphoid leukemias with aberrant expression of myeloid markers (ALL + M) [11–13]. In this type of leukemia the total score for the lymphoid markers is >2 whereas that for myeloid-associated markers is <2 (Figure 13.4). In our experience the most frequent aberrant myeloid markers expressed by ALL cells are CD13, CD15, and CD33. Other frequently reported myeloid markers in ALLs include CD14, CD65, and CD66c.
- C. Acute B-cell lymphoid leukemias with aberrant expression of T-cell markers (B-ALL + T) or acute T-cell lymphoid leukemias with aberrant expression of B-cell markers (T-ALL + B) [14].

TABLE 13.1 Scoring system for biphenotypic acute leukemias.*

Score	B-lymphoid	T-lymphoid	Myeloid
2	cyCD79a cylgM cyCD22	CD3 (s/cy) Anti-TCR	MPO
1	CD19 CD20 CD10	CD2 CD5 CD8 CD10	CD117 CD13 CD33 CD65
0.5	TdT CD24	TdT CD7 CD1a	CD14 CD15 CD64

*Adapted from Ref. [5] and European Group for the Immunologic Classification of Leukemia (EGIL).

cy: cytoplasmic; s: surface.

TABLE 13.2 Acute leukemias with aberrant markers, biphenotypic features, or bilineal populations.

1	Acute leukemias with aberrant expression: <ol style="list-style-type: none"> a. AMLs expressing lymphoid-associated markers (AML + L) such as CD7, CD19, and CD56 b. ALLs expressing myeloid-associated markers (ALL + M) such as CD13, CD15, and CD33
2	Acute biphenotypic leukemias: One population of leukemic cells co-expressing markers of both myeloid and lymphoid lineages or both B- and T-cells with scores of >2 for each lineage
3	Acute bilineal (mixed lineage) leukemias: Two separate populations of leukemic cells representing two distinct lineages

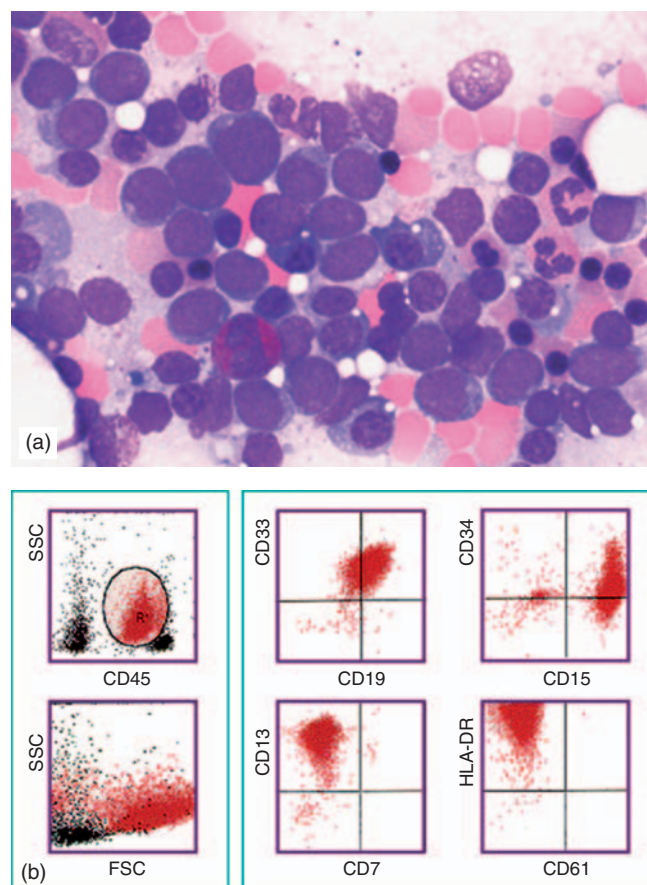


FIGURE 13.3 Acute myeloid leukemia with aberrant expression of CD19. (a) Bone marrow smear showing numerous blast cells and (b) flow cytometry revealing a population of CD13⁺, CD15⁺, CD33⁺, and CD34⁺ cells with aberrant expression of CD19.

Biphenotypic Acute Leukemia

In biphenotypic acute leukemia the population of leukemic cells co-expresses both myeloid and lymphoid or both B- and T-cell markers (Figures 13.5 and 13.6). The immunophenotypic score for each lineage is >2 [7, 15–19] (Table 13.1). Occasionally, the leukemic population may demonstrate a trilineage immunophenotypic picture, co-expressing a combination of myeloid, B-, and T-cell markers (triphenotypic) [20].

Bilineal Acute Leukemia

In bilineal acute leukemia two distinct populations of blast cells are present, each population representing a distinct lineage association. Most of the bilineal acute leukemias consist of two populations of myeloblasts and lymphoblasts, but rarely they may display a mixture of B- and T-lymphoblasts (Figures 13.7 and 13.8). The presence of two populations of blast cells in bilineal acute leukemia is often synchronous; both populations of blast cells are present at the same time. On rare occasions, there may be two simultaneous neoplastic processes in two separate sites. For example, the bone marrow may be involved with ALL and the CNS is infiltrated by AML [21].

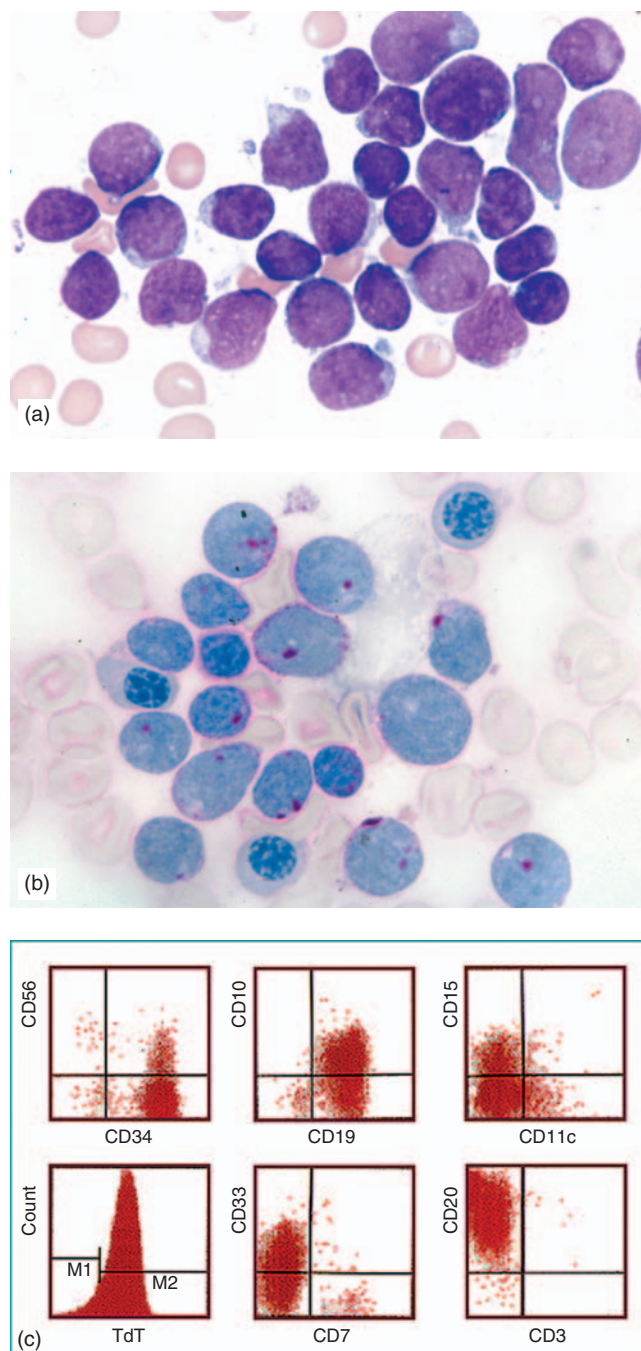


FIGURE 13.4 Acute lymphoid leukemia with aberrant expression of CD33. (a) Bone marrow smear showing numerous blast cells, (b) blasts containing coarse PAS⁺ cytoplasmic granules, and (c) flow cytometry revealing a population of CD10⁺, CD19⁺, CD20⁺, CD34⁺, and TdT⁺ cells with aberrant expression of CD15 (partial) and CD33.

Asynchronous bilineal acute leukemias are more frequently reported, and in those cases, one lineage switches to another during the disease process [22–29]. The predominant pattern in most studies is ALL switching to AML in relapse. The frequency of lineage switch in relapse is about 7%. Lineage switch may represent relapse of the original

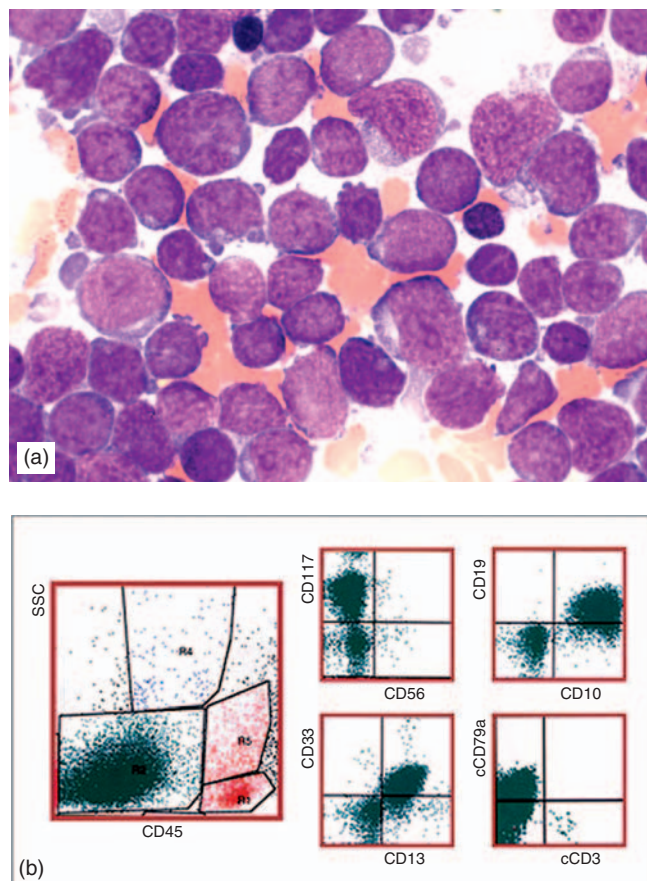


FIGURE 13.5 Biphenotypic acute leukemia. (a) Bone marrow smear demonstrating numerous pleomorphic blast cells with round or irregular nuclei and fine chromatin and (b) flow cytometry showing a population of CD45-negative cells co-expressing myeloid and B-cell markers.

clone or development of a second new clone. An increased risk of the development of AML has been reported in patients with ALL who receive intensive chemotherapy, particularly in patients with 11q23 abnormalities or the Philadelphia chromosome.

There are also occasional reports of switching from bilineal to biphenotypic acute leukemias and *vice versa* [30].

Cytochemical stains such as MPO, Sudan Black B, and NSE are helpful in distinguishing blasts of myeloid origin. Myeloblasts are often MPO and Sudan Black B positive and monoblasts/promonocytes usually express NSE.

Molecular and Cytogenetic Studies

IgH and *TCRγ* gene rearrangements and cyclin A1 and *HXA9* gene expression have been reported in biphenotypic acute leukemias [31]. Translocation of 11q23 and the Philadelphia chromosome are the most frequent cytogenetic abnormalities observed in biphenotypic and bilineal acute leukemias of B-precursor/myeloid type. More than 30 partner genes have been identified in association with 11q23 translocations such as t(4;11), t(9;11), and t(11;19) [3, 28, 32,

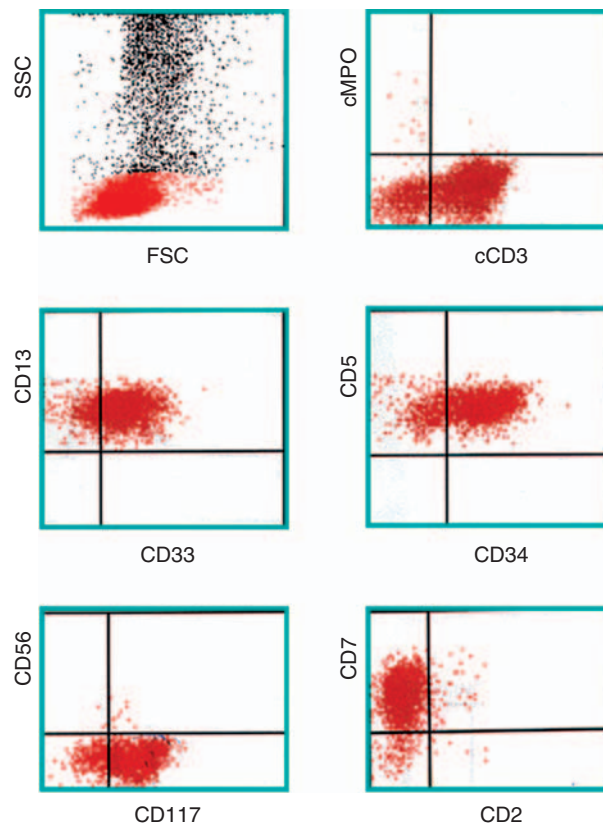


FIGURE 13.6 Flow cytometric analysis of a case of acute biphenotypic leukemia demonstrating CD34 positivity and co-expression of myeloid and T-cell markers.

33]. The Philadelphia chromosome may be a part of complex cytogenetic abnormalities, such as combination of t(9;22) and del(7) or t(2;9;22) (Figure 13.9) [34]. The T-precursor/myeloid biphenotypic or bilineal acute leukemias may be associated with t(5;18)(q31;q23) and t(3;12)(p25;q24.3) [35, 36]. Also, trisomy 10 has been reported in association with acute biphenotypic leukemia [37].

CLINICAL ASPECTS

Biphenotypic acute leukemias probably represent 4–8% of all acute leukemias [20]. The bilineal acute leukemias are less frequent. They occur at any age, but are more frequent in adults. Most of these leukemias are of precursor B-cell/myeloid type. They usually have an aggressive clinical course, probably due to the frequency of unfavorable karyotypes such as 11q23 abnormalities or the Philadelphia chromosome [38, 39]. These leukemias often show low complete remission rate, high recurrence rate, and short disease-free and overall survival time (Table 13.3).

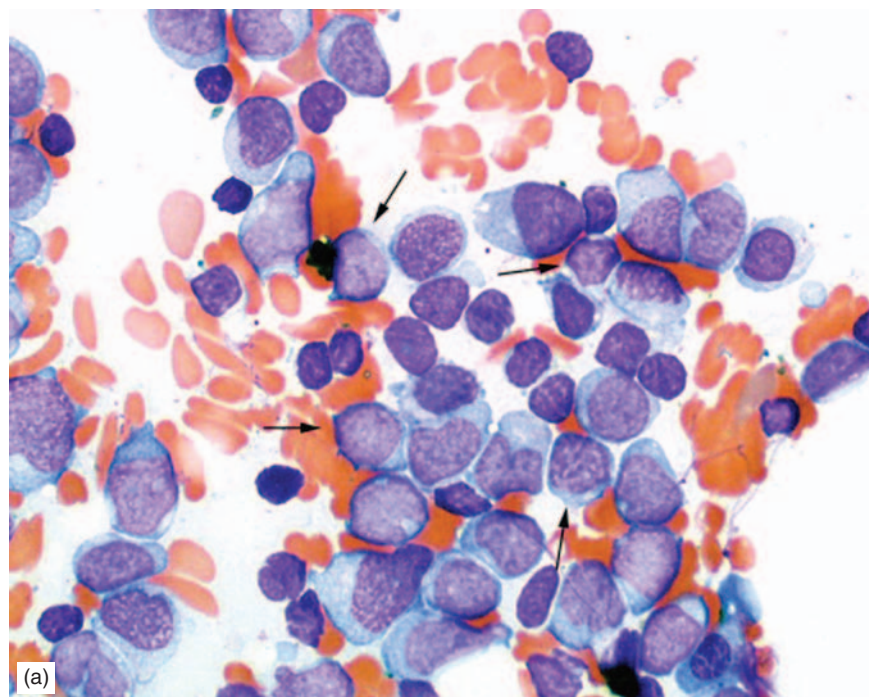
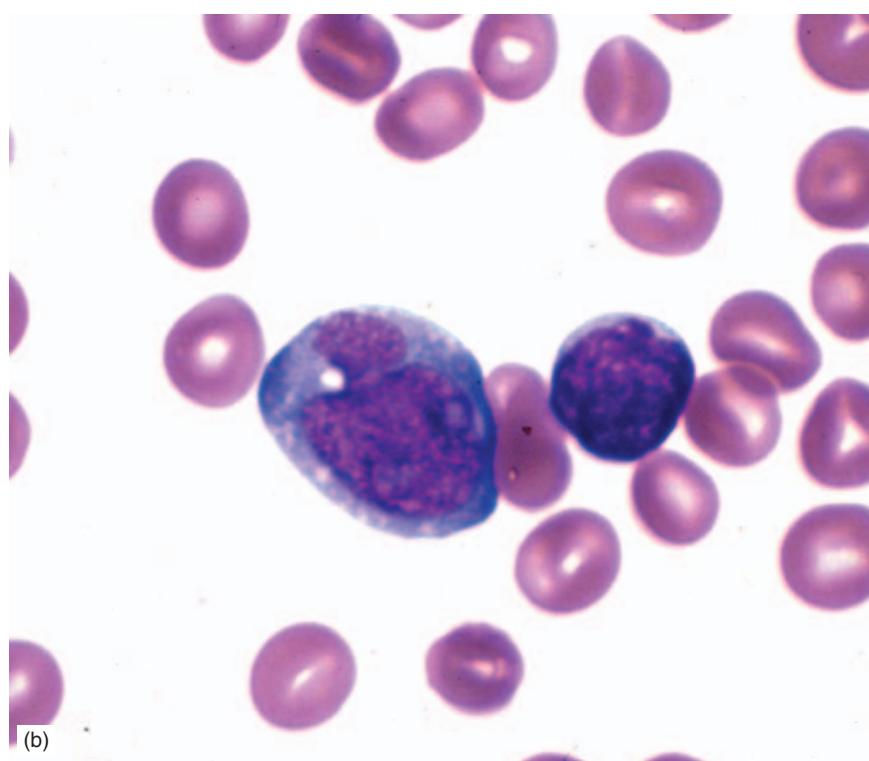


FIGURE 13.7 Acute bilineal leukemia. Bone marrow smear showing scattered small blasts with round nuclei and scanty cytoplasm (arrows) and numerous larger blasts with round or irregular nuclei and abundant cytoplasm (a). Blood smears revealing a small blast with scanty cytoplasm and inconspicuous nucleoli and a larger blast with abundant cytoplasm, irregular nucleus, and prominent nucleoli (b).



DIFFERENTIAL DIAGNOSIS

The diagnosis of biphenotypic acute leukemia is established by immunophenotypic studies. These leukemias should be distinguished from AMLs with aberrant expression of lymphoid-associated markers (AML + L) and ALLs with aberrant expression of myeloid-associated markers (ALL + L).

Bilineal acute leukemias may show morphologic evidence of two separate leukemia populations such as larger and smaller blasts. But diagnosis is confirmed by the evidence of two populations of blast cells distinctly expressing different lineage-associated markers by immunophenotypic studies.

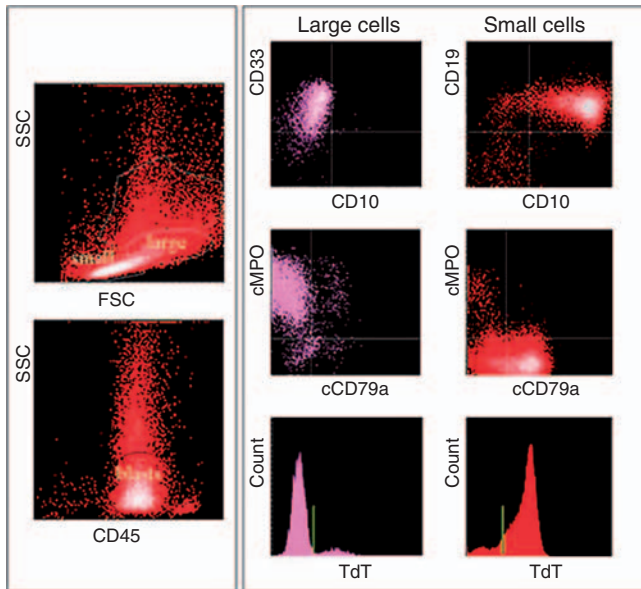


FIGURE 13.8 Acute bilineal leukemia. Flow cytometric studies revealing two distinct populations of larger and smaller blast cells with immunophenotypic features consistent with acute myeloid and acute lymphoid leukemias, respectively.

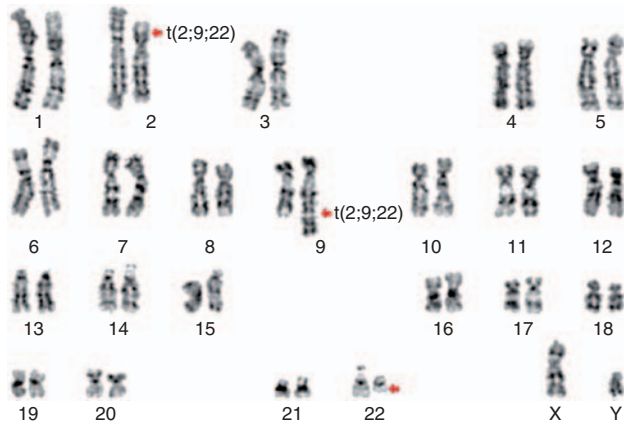


FIGURE 13.9 G-banded karyotype in a patient with CML in blast transformation. There is a three-way translocation involving chromosomes 2, 9, and 22 (arrows).

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TABLE 13.3 Clinical and cytogenetic features of biphenotypic acute leukemias.

Clinical features
About 4–8% of acute leukemias
Poor prognosis
Low rate of complete remission
High recurrence rate
Short disease-free and overall survival time
Cytogenetic abnormalities
Precursor B-cell/myeloid
t(9;22), 11q23 (MLL) abnormalities
Precursor T-cell/myeloid
t(8;13), t(3;12)
Other less frequent abnormalities
14q32, t(3;7), t(5;18), t(6;14), t(12;17)

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Lymphoid Malignancies of Non-precursor Cells: General Considerations

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In the following chapters, all categories of lymphoid malignancies except those of precursor B- and T-cell lymphoblastic leukemias/lymphomas are discussed. These tumors, according to the WHO classification, are divided into the following major categories [1]:

1. Mature B-cell neoplasms
2. Mature T- and NK-cell neoplasms
3. Hodgkin lymphoma.

Mature B-cell neoplasms comprise a wide spectrum of lymphoid malignancies representing clonal proliferation of B-lymphocytes at various stages of differentiation, from the early naïve B-cells to the end-stage mature plasma cells. These disorders may primarily involve bone marrow and peripheral blood (leukemia), lymphoid or extramedullary tissues (lymphoma), or both. They comprise >85% of all lymphoid neoplasms and are divided into numerous categories [1, 2]. The most frequent types are diffuse large B-cell lymphoma and follicular lymphoma accounting for about 30% and 20%, respectively, of all non-Hodgkin lymphoid malignancies.

Mature T-cell neoplasms represent the post-thymic stages of maturation. These tumors, along with NK-cell neoplasms, account for >15% of all lymphoid malignancies [1, 2]. Similar to the mature B-cell neoplasms, T- and NK-cell malignancies may primarily involve bone marrow and peripheral blood (leukemia), lymphoid or extramedullary tissues (lymphoma), or more commonly both.

Hodgkin lymphoma represents a heterogeneous but distinct clinicopathologic entity characterized by the presence of neoplastic Reed-Sternberg cells and their variants in a background of an admixture of reactive inflammatory cells,

preferential involvement of lymph nodes, and frequent occurrence in young adults. In most instances, the neoplastic cells appear to be of B-cell lineage. Hodgkin lymphoma comprises about 30% of all lymphomas and is divided into two major categories: (1) nodular-lymphocyte-predominant and (2) classical Hodgkin lymphomas [3].

This chapter presents an overview of non-Hodgkin and Hodgkin lymphoid malignancies.

ETIOLOGY AND PATHOGENESIS

The etiology of lymphomas is not fully understood. A number of environmental factors are associated with increased incidence of lymphomas. An elevated risk of the development of non-Hodgkin lymphoma has been associated with exposure to pesticides, fertilizers, organic solvents, epoxy glues, and wood dust [4–6]. Ultraviolet light and hair dyes have also been implicated in some studies [4].

A number of viruses, such as Epstein-Barr virus (EBV), human T-cell lymphotropic virus (HTLV-1), and human herpes virus-8 (HHV-8), play a crucial role in the development of certain categories of lymphomas [7–14]. Epstein-Barr virus has been detected in endemic African and in a fraction of sporadic forms of Burkitt lymphomas. Also, association of EBV infection with B-cell lymphomas has been observed in immunosuppressive conditions, such as AIDS, and in immunosuppressive therapy after organ transplantation [8–12]. Infection of EBV in lymphoid tissue is frequently documented in a significant proportion of cases with classical Hodgkin lymphoma. The EBV genome in the

infected lymphoid cells usually exists as an extrachromosomal circular episome in the nucleus. The EBV infection promotes lymphocytic proliferation with clonal expansion of the EBV genome. The lymphocytic proliferation, in certain conditions, may increase the chance of chromosomal aberrations. HTLV-1 has been detected in 100% of the cases of adult T-cell leukemia/lymphoma. The HTLV-1 transregulatory product, HTLV-1-Tax, activates certain host genes such as IL-2, PDGF, GM-CSF, and cyclin-dependent kinase inhibitor p21/waf1 [13, 14]. Human herpes virus-8 has been detected in the primary effusion lymphomas in virtually 100% of the cases [15]. The mechanism of viral carcinogenesis in the primary effusion lymphomas is not fully understood. There is also a strong association between gastric mucosa-associated lymphoid tissues (MALT) type lymphoma and *Helicobacter pylori* [16–18].

Lymphomagenesis appears to be a multistep process associated with a number of genetic changes affecting proto-oncogenes and tumor suppressor genes [19–22]. The cytogenetic alterations in lymphoma cells are of two types: balanced and unbalanced chromosomal changes [23].

Reciprocal chromosomal translocations are the characteristic features of lymphoid malignancies. Translocations in non-Hodgkin lymphoma of B-cell types involve chromosomal breakpoints within immunoglobulin (Ig) loci while T-cell receptor (TCR) loci are commonly implicated in T-cell lymphomas. Breakpoints within the Ig loci are often located within the joining (J) and switch (S) sequences. Chromosomal translocations in non-Hodgkin lymphoma, in addition to Ig or TCR loci, involve proto-oncogenes in proximity to the chromosomal recombination sites. Usually, in these translocations, the structure of the proto-oncogene is not affected. The juxtaposition of the regulatory DNA sequences derived from the partner chromosome changes the pattern of expression of the oncogene. Less commonly, in

certain types of lymphomas, such as anaplastic large cell and MALT, the chromosomal translocation results in the juxtaposition of two genes, creating a chimeric gene coding for a new protein [24, 25].

Unbalanced chromosomal abnormalities are usually preceded by balanced translocations and appear to represent the second genetic hit in the clonal evolution of the lymphoid malignancies. For example, in addition to t(14;18), loss of 6q has been described in follicular lymphomas and appears to predict poor prognosis [26].

Inactivation of tumor suppressor genes has been observed in some lymphomas and is usually the result of point mutation of one allele and/or chromosomal deletion of the second allele. Deletions and mutations of the *p53* tumor suppressor gene have been associated with chronic lymphocytic leukemia or small lymphocytic, follicular, Burkitt, and mantle cell lymphomas [27–31].

PATHOLOGY

Morphology

Lymphoid malignancies have extremely diverse morphologic features. This diversity, to some degree, correlates with the stage of their differentiation. The precursor B-cell goes through several maturation and evolutionary steps, such as naïve lymphocyte, mantle cell, follicular B-blast, centroblast, centrocyte, marginal zone B-cell, B-immunoblast, and plasma cell, and each step depicts some characteristic morphologic features (Figure 14.1). The non-Hodgkin lymphoid malignancies, particularly of B-cell types, show two major patterns of lymph node involvement: follicular and diffuse (Figure 14.2).

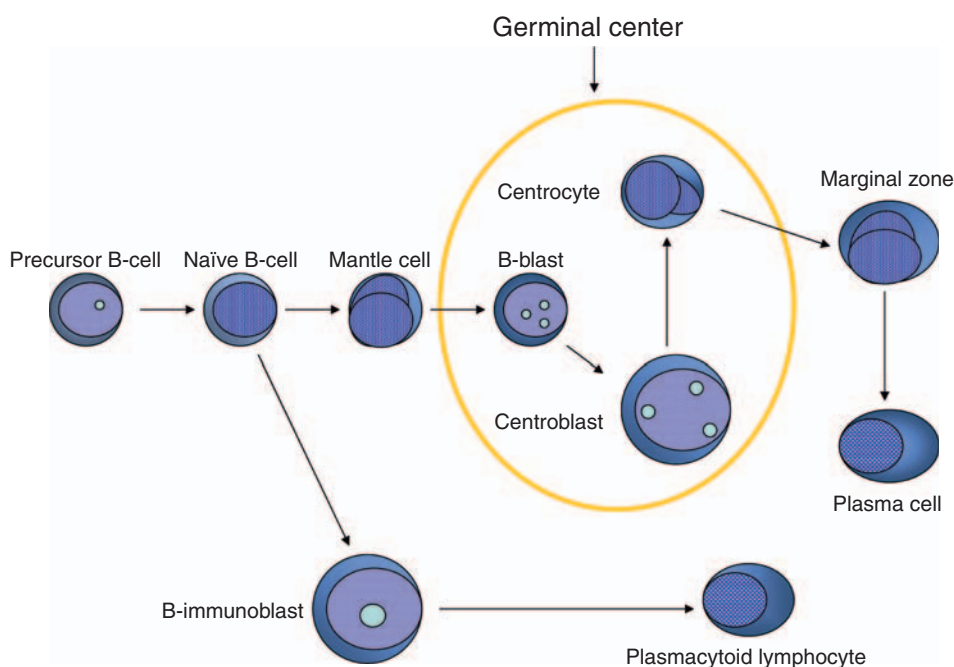


FIGURE 14.1 Scheme of B-cell differentiation. Adapted from Harris NL, Ref. [1].

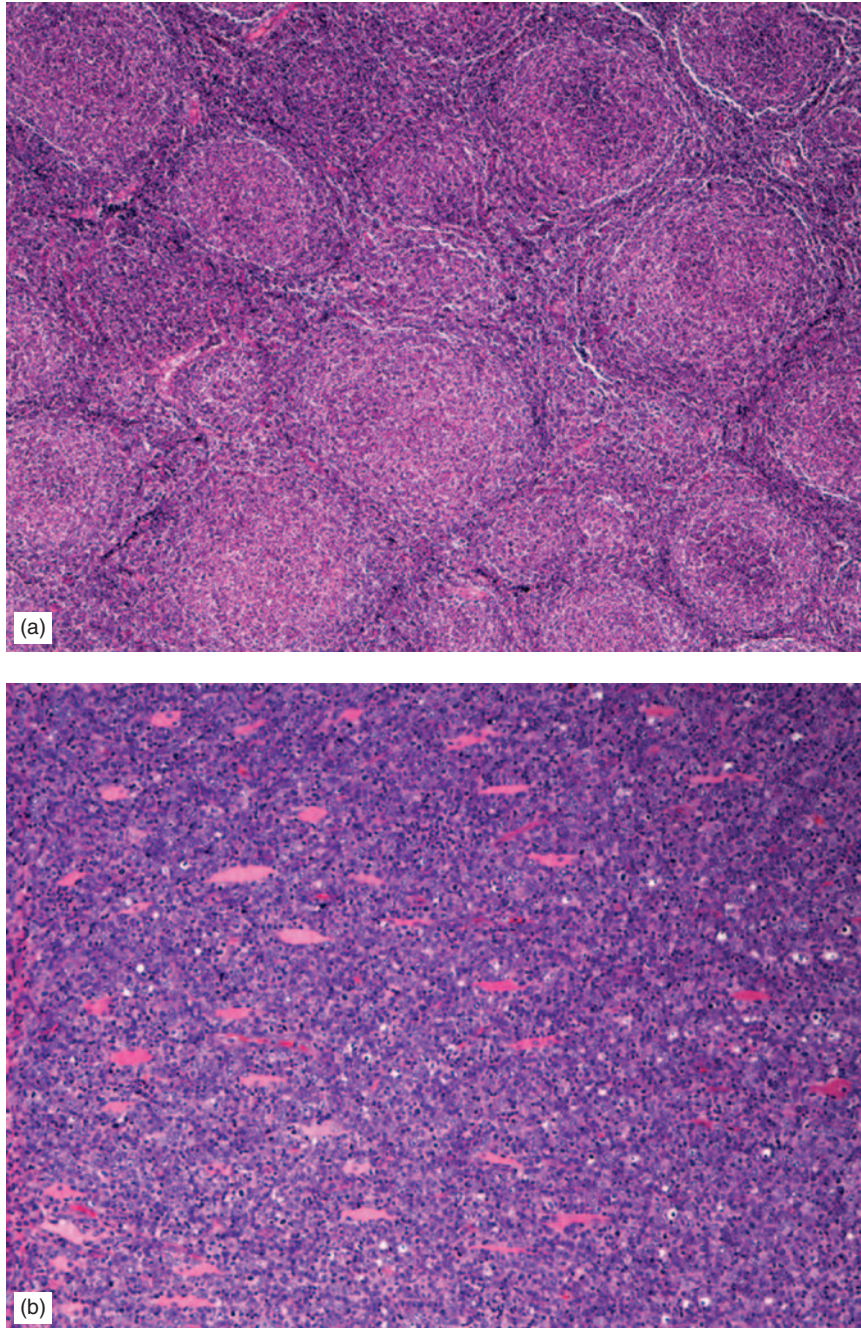


FIGURE 14.2 Lymph node sections demonstrating follicular (a) and diffuse (b) patterns of lymphoma.

The T-cell lymphoid malignancies are virtually all diffuse. They also comprise a diverse morphology ranging from small lymphocytes to large, bizarre anaplastic tumor cells. The NK-cell neoplasms usually show cytoplasmic granules and represent a subgroup of large granular lymphocytes (LGLs). Large granular lymphocytes are divided into two major categories: T- and NK-cell types. These two categories are morphologically indistinguishable, but one (T-cell type) demonstrates *TCR* gene rearrangement and the other (NK-cell type) does not (Figure 14.3).

Bone marrow involvement in the mature B- and T-cell neoplasms may appear in different patterns such as diffuse, paratrabeular, nodular, interstitial, or mixed. Diffuse

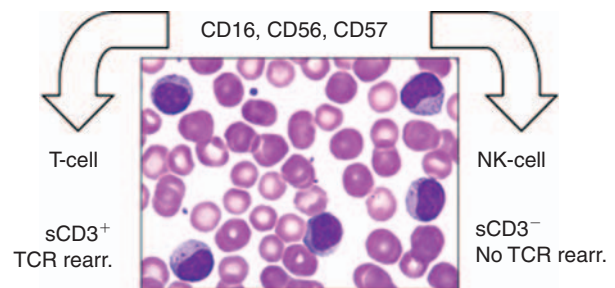


FIGURE 14.3 Large granular lymphocytes (LGLs) are of two types: T- and NK-cells. T-cell LGLs express surface CD3 (sCD3) and demonstrate T-cell receptor (TCR) gene rearrangement. NK-cell LGLs are negative for sCD3 and do not show *TCR* gene rearrangement.

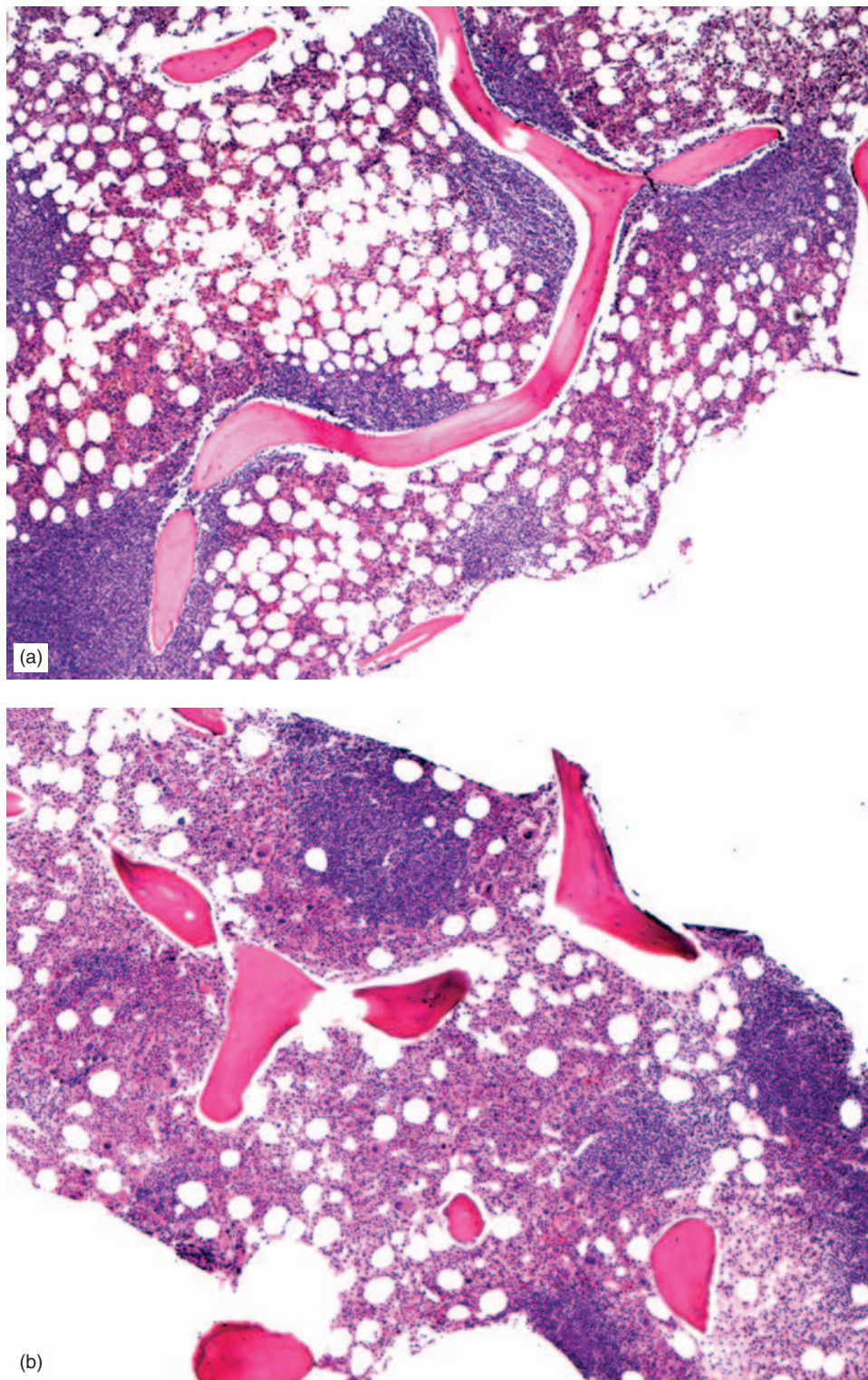


FIGURE 14.4 Bone marrow biopsy sections showing paratrabecular (a) and nodular (b) lymphomatous involvement.

involvement is defined as sheets of space-occupying lymphoid cells without the formation of well-defined nodular aggregates. Paratrabecular disease refers to aggregates of lymphoma cells next to the bony trabeculae (Figure 14.4a). Nodular involvement consists of well-defined non-paratrabecular aggregates (nodules) of lymphoma cells (Figure 14.4b) [32]. Interstitial involvement is demonstrated by

infiltration of lymphoma cells into the fatty tissue without obvious obliteration of the bone marrow architecture on low-power examination (Figure 14.5).

The morphologic diversity in Hodgkin lymphomas is the result of both the number and the type of Reed–Sternberg and Hodgkin cells as well as the proportion of the lymphocytic component in the background of mixed

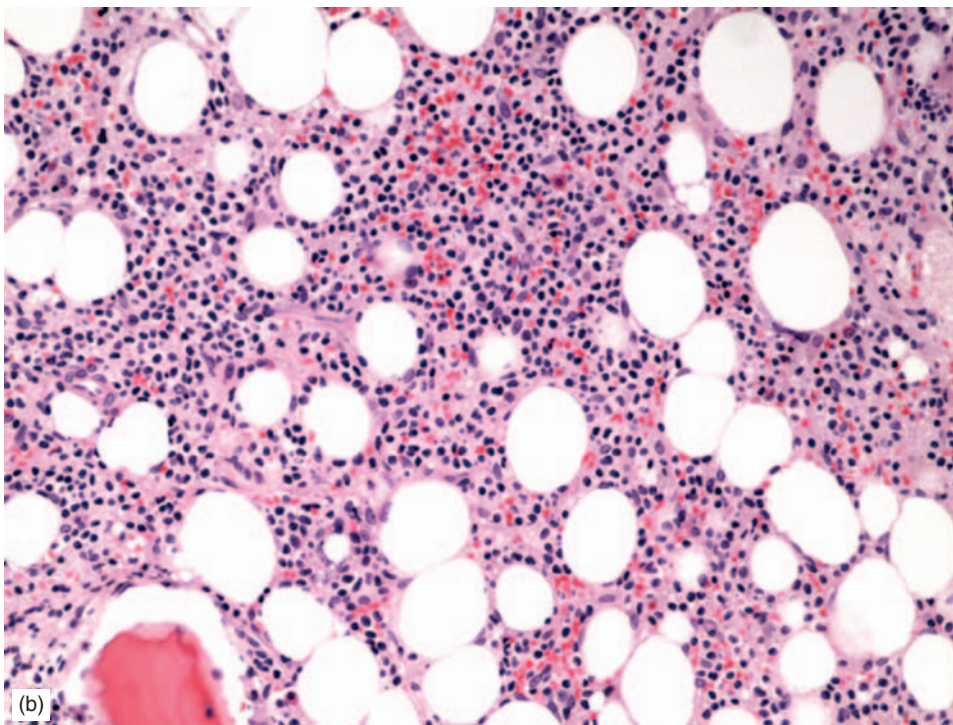
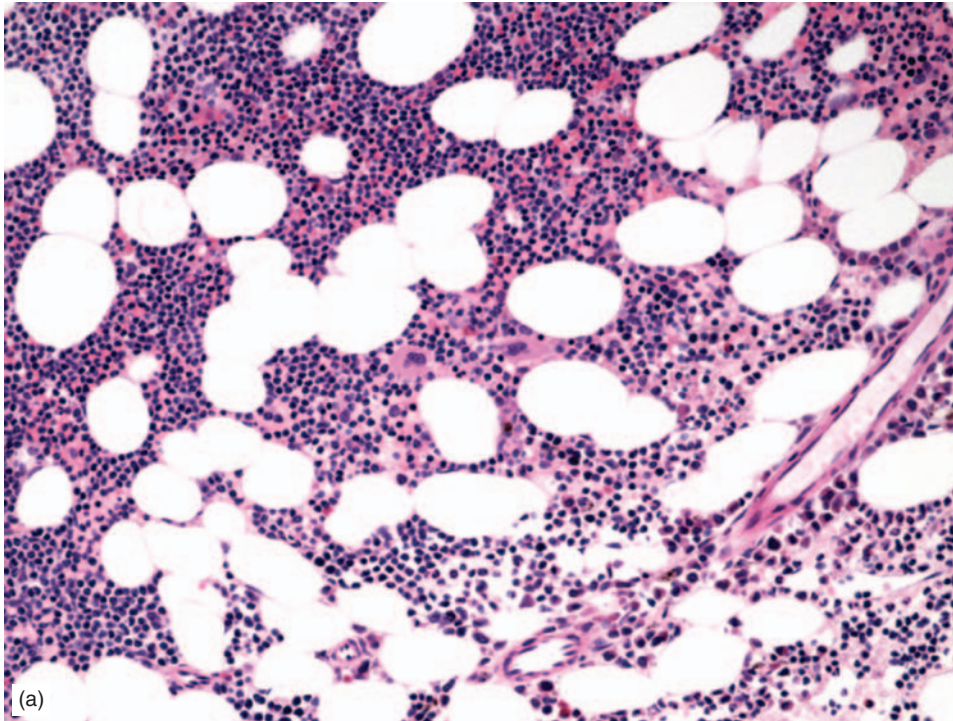


FIGURE 14.5 Bone marrow biopsy sections showing interstitial involvement in patients with chronic lymphocytic (a) and hairy cell leukemias (b).

inflammatory cells. Involvement of bone marrow in Hodgkin lymphoma is significantly less frequent than that in non-Hodgkin lymphoma. This involvement is often nodular and is associated with variable degrees of fibrosis.

The WHO classification of non-Hodgkin lymphoid malignancies is primarily based on morphologic and

immunophenotypic features of the neoplastic cells (Table 14.1) [1], whereas that of Hodgkin lymphoma is based on morphologic features (Table 14.1).

Mature B-cell and T-cell lymphoid malignancies, plasma cell neoplasms, and Hodgkin lymphomas are discussed separately in Chapters 15, 16, and 17, respectively.

TABLE 14.1 The WHO classification of mature lymphoid malignancies and Hodgkin lymphoma.*

Mature B-cell
Chronic lymphocytic leukemia/small lymphocytic lymphoma
B-cell prolymphocytic leukemia
Lymphoplasmacytic lymphoma
Splenic marginal zone lymphoma
Hairy cell leukemia
Plasma cell neoplasms
Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue
Nodal marginal zone B-cell lymphoma
Follicular lymphoma
Mantle cell lymphoma
Diffuse large B-cell lymphoma
Mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
Primary effusion lymphoma
Burkitt lymphoma/leukemia
Mature T- and NK-cells
Leukemic/disseminated
T-cell prolymphocytic leukemia
T-cell large granular lymphocytic leukemia
Aggressive NK-cell leukemia
Adult T-cell leukemia/lymphoma
Cutaneous
Mycosis fungoides
Sezary syndrome
Primary cutaneous anaplastic large cell lymphoma
Lymphomatoid populosus
Other extranodal
Extranodal NK/T-lymphoma, nasal type
Entropathy-type T-cell lymphoma
Hepatosplenic T-cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma
Nodal
Angioimmunoblastic T-cell lymphoma
Peripheral T-cell lymphoma, unspecified
Anaplastic large cell lymphoma
Hodgkin lymphoma
Nodular-lymphocyte-predominant Hodgkin lymphoma
Classical Hodgkin lymphoma
Nodular sclerosis classical Hodgkin lymphoma
Mixed cellularity classical Hodgkin lymphoma
Lymphocyte-rich classical Hodgkin lymphoma
Lymphocyte-depleted classical Hodgkin lymphoma

*Adapted from Ref. [1].

TABLE 14.2 Major characteristic immunophenotypic features in small mature B-cell lymphoid malignancies.

Type of lymphoid malignancy	Immunophenotype	
	Positive	Negative
CLL/SLL	CD5, CD19, CD23	CD10, FMC7, CD103, bcl-1
Mantle cell	CD5, CD19, CD43, FMC7, bcl-1	CD10, CD23, bcl-6
Marginal zone	CD19, CD29, CD79a	CD5, CD10, CD23, bcl-1
Follicular	CD10, CD19, CD20, bcl-2, bcl-6	CD5, CD43, bcl-1
Lymphoplasmacytic	CD19, CD20, CD22, CD79a	CD5, CD43, bcl-1
Hairy cell	CD19, CD11c, FMC7, CD22, CD103	CD5, CD10, CD23, bcl-1
Prolymphocytic	CD19, CD20, FMC7, CD22, CD79a	CD23, CD10, and usually CD5
Burkitt	CD10, CD19, CD20, CD22, bcl-6, KI-67	CD5, CD23, CD34, TdT, bcl-2

Immunophenotype

The mature B-cell neoplasms are negative for CD34 and TdT and express various B-cell-associated antigens according to their stage of maturation (Table 14.2). For example, neoplastic cells in chronic lymphocytic leukemia or small lymphocytic lymphoma correspond to naïve B-cells and co-express CD5, CD19, CD20, and CD23; whereas lymphoid cells in mantle cell lymphoma are positive for CD5, CD19, and CD20, but negative for CD23. Neoplasms of follicular-center-cell origin express CD10, but are negative for CD5; marginal zone B-cell lymphomas do not express CD5, CD10, and CD23.

The mature T- and NK-cell neoplasms are also negative for CD34 and TdT. The mature T-cell neoplasms express the entire TCR-CD3 complex and show both cytoplasmic and surface positivity for CD3. These tumors are mostly of the helper T-cell type and therefore express CD4. NK-cell neoplasms express only the ϵ chain of CD3 in the cytoplasm; they are negative for CD4, but may express CD8. Both T- and NK-cells express CD2 and CD7. The large granular cells of T- or NK-cell origin commonly express CD16, CD56, and/or CD57 and show positive reactions for cytotoxic proteins such as perforin, TIA-1, and granzyme B (see Figure 14.3).

The immunophenotypic features of Reed–Sternberg cells and their variants are different in nodular-lymphocyte-predominant and classical Hodgkin lymphomas. These cells in nodular-lymphocyte-predominant Hodgkin lymphoma are positive for CD45 and often express B-cell-associated markers such as CD20 and CD79a, but are negative for CD15 and CD30. In classical Hodgkin lymphoma,

TABLE 14.3 Major characteristic cytogenetic features of small mature B-cell lymphoid malignancies.

Type of lymphoid malignancy	Chromosomal abnormalities	Genes
CLL/SLL	del(17)(p13) del(11)(q22) Trisomy 12 del(13)(q14)	<i>p53</i> <i>ATM</i>
Mantle cell	t(11;14)(q13;q32)	<i>BCL-1/IGH</i>
Marginal zone Splenic Extranodal	del(7q) Trisomy 3 t(11;18)(q21;q21)	<i>BIRC3/MALT1</i>
Follicular	t(14;18)(q32;q21)	<i>IGH/BCL-2</i>
Lymphoplasmacytic	del(6)(q21→q23) t(9;14)(p13;q32)	<i>PAX5/IGH</i>
Hairy cell	Non-specific	
B-prolymphocytic	14q32 abnormalities	<i>IGH</i>
Burkitt	t(8;14)(q24;q32) t(2;8)(p12;q24) t(8;22)(q24;q11.2)	<i>cMYC/IGH</i> <i>IGK/cMYC</i> <i>cMYC/IGL</i>

Reed–Sternberg cells and their variants are negative for CD45 and express CD15 and CD30.

Cytogenetic and Molecular Studies

The hallmark of mature lymphoid malignancies is reciprocal chromosomal translocation. In most translocations, an intact proto-oncogene is located next to the *IGH* or *TCR* genes. Quantitative chromosomal changes such as trisomies and deletions also occur, which in some cases involve tumor suppressor genes. As an example, Table 14.3 demonstrates the major characteristic cytogenetic features observed in mature small B-cell lymphoid neoplasms [23].

The most common proto-oncogenes involved in B-cell neoplasms are *BCL1* (*CCND1*), *BCL-2*, *BCL-6*, *MYC*, and *PAX-5* [33–35]. Of these, the one that is most often tested at the molecular level is *BCL-2*. The others are either too uncommon or too heterogeneous in their molecular breakpoints to be efficiently detected by PCR or Southern blot and are better addressed by karyotype or fluorescence *in situ* hybridization (FISH) to detect the translocation, or by immunohistochemistry to detect increased expression of the protein product. Similar to the *BCR-ABL* translocation discussed in Chapter 11, the t(14;18) translocation of *BCL-2* may be detected by either Southern blot or PCR. For the latter, direct DNA-based PCR is adequate, unlike *BCR-ABL* testing which requires RT-PCR. The *ALK* and *NFKB-2* genes are involved in certain types of T-cell malignancies such as anaplastic large cell and cutaneous T-cell lymphomas, respectively [19, 34, 35]. The most

common tumor suppressor gene involved in lymphoid malignancies is *p53*. A large proportion of molecular changes in lymphoid malignancies are readily and inexpensively detected at the protein level (immunohistochemistry and/or flow cytometry) than molecular techniques.

The mature B-cell and plasma-cell disorders naturally demonstrate clonal immunoglobulin gene rearrangements, but since they are usually readily diagnosed by routine means, molecular analysis is not often necessary. As the T-cell malignancies may have fewer protein markers to choose from, clonality studies may be helpful to both diagnose and differentiate them from B-cell lesions. *ALK* is an important alternative target in some cases of T-cell malignancy, but again it is usually easier to detect overexpression of the protein by immunochemical means, or to detect the common t(2;5) translocation or the variants by an appropriate FISH probe, which is actually more sensitive than PCR [36]. As for Hodgkin disease, although gene rearrangement studies have been invaluable in research to prove the B-cell origin of this tumor, the malignant cells (Reed–Sternberg) are usually too scanty in the specimen to produce a robust clonal signal in routine analysis [37].

The more detailed cytogenetic and molecular characteristics of specific subtypes of the lymphoid neoplasms with illustrations are presented in Chapters 15 through 18.

CLINICAL ASPECTS

The lymphoid neoplasms of non-precursor cells represent the fifth most common malignancy in the United States accounting for about 4% of all cancers. They are more common in men than in women and are more frequent in adults than in children with a steady increase in incidence with age. In general, the lymphoid malignancies in children are more commonly extranodal than nodal and clinically more aggressive. The overwhelming majority of lymphoid malignancies are of B-cell lineage accounting for >85% of the cases. All types of T/NK-cell neoplasms make up about 15% of the total lymphoid malignancies (Table 14.4).

The Ann Arbor staging system originally developed for the staging of Hodgkin lymphomas has been extended to non-Hodgkin lymphoid malignancies. This staging system is based on the location(s), number of involved sites, and presence or absence of systemic symptoms (Table 14.5). Since in the majority of cases of non-Hodgkin lymphoma the disease is disseminated at the time of diagnosis, the staging system is less useful in non-Hodgkin lymphoma than it is in Hodgkin lymphoma.

An international prognostic index (IPI) has been proposed for patients with non-Hodgkin lymphoma. This scoring system is based on the following factors, which were found to show a reverse correlation with relapse-free survival [38]:

Age >60 years

Elevated serum lactate dehydrogenase (LDH)

TABLE 14.4 Frequency of non-Hodgkin lymphoid malignancies.^a

Type of lymphoma	Approximate frequency (%)
Diffuse large B-cell	31
Follicular	22
MALT	8
Mature T-cell (except ALCL ^b)	8
CLL/SLL ^c	7
Mantle cell	6
Mediastinal large B-cell	2.5
ALCL	2.5
Burkitt	2.5
Nodal marginal zone	2
Lymphoplasmacytic	1
Others	7.5

^aAdapted from Refs. [1, 38].

^bAnaplastic large cell lymphoma.

^cChronic lymphocytic leukemia/small lymphocytic lymphoma.

TABLE 14.5 The Ann Arbor staging for lymphomas.

Stage I	Involvement of a single node region or a single extralymphatic organ or site (Stage 1E).
Stage II	Two or more involved lymph node regions on the same side of the diaphragm, or with localized involvement of an extralymphatic organ or site (IIe).
Stage III	Lymph node involvement on both sides of the diaphragm, or with localized involvement of an extralymphatic organ or site (IIe), or spleen (IIIS), or both (IIIES).
Stage IV	Presence of diffuse or disseminated involvement of one or more extralymphatic organs, with or without associated lymph node involvement.
Systemic symptoms	
A	Asymptomatic.
B	Presence of fever, sweats, or weight loss >10% of body weight.

Eastern Cooperative Oncology Group (ECOG) performance status >2

Ann Arbor clinical stage III or IV

Number of involved extranodal disease sites >1.

Each factor receives a score of 1. Non-Hodgkin lymphomas with a total IPI score of 0–1 are considered low risk, and those with IPI scores of 2, 3, and >3 are in the

TABLE 14.6 The prognostic categories for lymphoid malignancies.*

B-cell lineage	T-cell lineage
<i>Indolent</i>	
CLL/SLL	LGL, T-cell type
Hairy cell leukemia	Mycosis fungoides and Sezary syndrome
Splenic marginal zone	Primary cutaneous anaplastic large cell
MALT type leukemia/lymphoma	Smoldering and chronic adult T-cell
Nodal marginal zone	
Follicular lymphoma, grades I and II	
Lymphoplasmacytic	
<i>Aggressive</i>	
Plasma cell myeloma	Prolymphocytic
Mantle cell	Peripheral T-cell, unspecified
Follicular lymphoma, grade III	Angioimmunoblastic
Diffuse large cell	T/NK-cell of nasal type
Primary mediastinal large cell	Anaplastic large cell lymphoma
	Hepatosplenic T-cell
	Subcutaneous panniculitis-like
<i>Highly aggressive</i>	
Precursor B-lymphoblastic	Precursor T-lymphoblastic
Burkitt	Adult T-cell leukemia/lymphoma

*Adapted from Ref. [39].

categories of low intermediate risk, high intermediate risk, and high risk, respectively.

A clinicopathologic prognostic scheme was proposed by Hiddemann *et al.* [39], in which the non-Hodgkin lymphoid malignancies were divided into three major groups of indolent, aggressive, and highly aggressive tumors (Table 14.6).

DIFFERENTIAL DIAGNOSIS

The presence of a wide variety of mature lymphoid malignancies and numerous subcategories makes their accurate diagnosis and classification very challenging. In spite of significant clarification in diagnostic criteria and classification, advances in technology, and availability of accessory tools in numerous diagnostic centers, there is still evidence of significant discordance in the diagnosis and classification of lymphoid malignancies among pathologists [40–42]. For example, in one study, the agreement rate in diagnosis/classification of lymphomas among the pathologists ranged from <50% in cases of Burkitt-like lymphoma and lymphoplasmacytic lymphoma to >85% in cases of small mature B-cell lymphoid malignancies, diffuse large B-cell lymphoma, and other lymphoid malignancies [40]. Some

general practical points helpful in differential diagnosis of mature lymphoid neoplasms are presented as follows:

1. Morphologic features in many instances are not sufficient to separate the B-cell from the T-cell disorders. Some subcategories of small mature B-cell malignancies, such as follicular lymphoma, marginal zone B-cell lymphoma, and mantle cell lymphoma, may morphologically mimic one another. Large granular lymphocytes of NK- and T-cell origins are morphologically indistinguishable. Therefore, it is highly recommended that immunophenotypic, cytogenetic (more specifically FISH), and sometimes molecular studies be part of the routine diagnostic work-up of lymphoid malignancies.
2. Node-based lymphomas should be distinguished from garden varieties of reactive lymphadenopathies such as follicular lymphoma from follicular hyperplasia, Hodgkin lymphoma from viral infections, and drug-induced lymphadenitis from T-cell lymphoma.
3. Cutaneous lymphoid malignancies may mimic various inflammatory dermal lesions.
4. Burkitt lymphoma/leukemia should be distinguished from precursor B- and T-cell lymphoblastic leukemias/lymphomas.
5. Anaplastic large cell and non-Hodgkin lymphomas share overlapping morphologic features.

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Mature B-Cell Neoplasms

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Mature B-cell neoplasms represent a wide spectrum of lymphoid malignancies developed from clonal proliferation of B-lymphocytes at various stages of differentiation from the early naïve B-cells to the end-stage mature plasma cells (Figure 15.1). These lymphoproliferative disorders may primarily involve bone marrow and peripheral blood (leukemia), lymphoid or extramedullary tissues (lymphoma), or both. They are more frequent than T-cell malignancies and comprise >85% of all lymphoid tumors [1, 2].

Mature B-cell neoplasms demonstrate clonal immunoglobulin gene rearrangements by either PCR or Southern blot. Unlike the precursor B-cell lesions, they will usually show rearrangements in their light chain genes as well as in the heavy chain genes, but since the IgH locus has become the standard screen for all B-cell clonality studies, it is easier to begin with this one from a laboratory management perspective. Any equivocal results can be confirmed by examining the light chain genes, most commonly J_K . As noted in Chapter 14, most of these cases are readily diagnosed by histologic and immunochemical methods, so gene rearrangement studies are usually not needed. However, they will be introduced in some detail here, since the basic principles of detection are the same for all the B-cell-derived lymphoid neoplasms to be covered subsequently.

The hallmark of B-cell leukemia/lymphoma diagnosis by gene rearrangement studies is the demonstration of clonal patterns of rearrangement in the DNA of the immunoglobulin loci. These rearrangements are a normal part of the maturation of all B-lymphocytes, as a means to expand the repertoire of produced antibody specificities, so that a finite number of genes can ultimately encode an almost limitless number of antibodies capable of responding to

the wide variety of epitopic insults to which the body is prone during life. Once a particular rearrangement occurs in an individual lymphocyte (typically on only one allele, the remaining is fixed by the principle of “allelic exclusion”), all the daughter cells of that lymphocyte will contain the same rearrangement pattern forming a subclone. Because of the tremendous number of possible rearrangements, the subclones in a normal or reactive (nonmalignant) specimen (blood, bone marrow, lymph node, etc.) will span the entire range of DNA patterns with no single subclone represented in a high enough percentage to be detectable by the standard methods of analysis. In contrast, most malignant lesions are assumed to be clonal, having descended from a transforming event in a single progenitor cell, and the specimen should therefore exhibit a single predominant rearrangement pattern, one present at sufficiently high proportion to stand out from the background polyclonal “smear.” It should be noted that very few specimens sent to the clinical laboratory are “pure” tumor; most will contain a background of incidental benign cells or, in the case of tissue biopsies (e.g. skin), a complement of nonlymphoid cells (e.g. fibroblasts) that do not undergo immunoglobulin gene rearrangements in any circumstances. These background cells, along with the typically unrearranged allele seen even in malignant lymphocytes (representing half the total target DNA), are responsible for the background polyclonal “smear” seen in both Southern blot and PCR assays and for the persistence of the “germline bands” seen on Southern blot (see examples in figures).

Although all of the immunoglobulin gene loci undergo rearrangement, convention in the field has coalesced around the detection of heavy chain rearrangements (located on chromosome

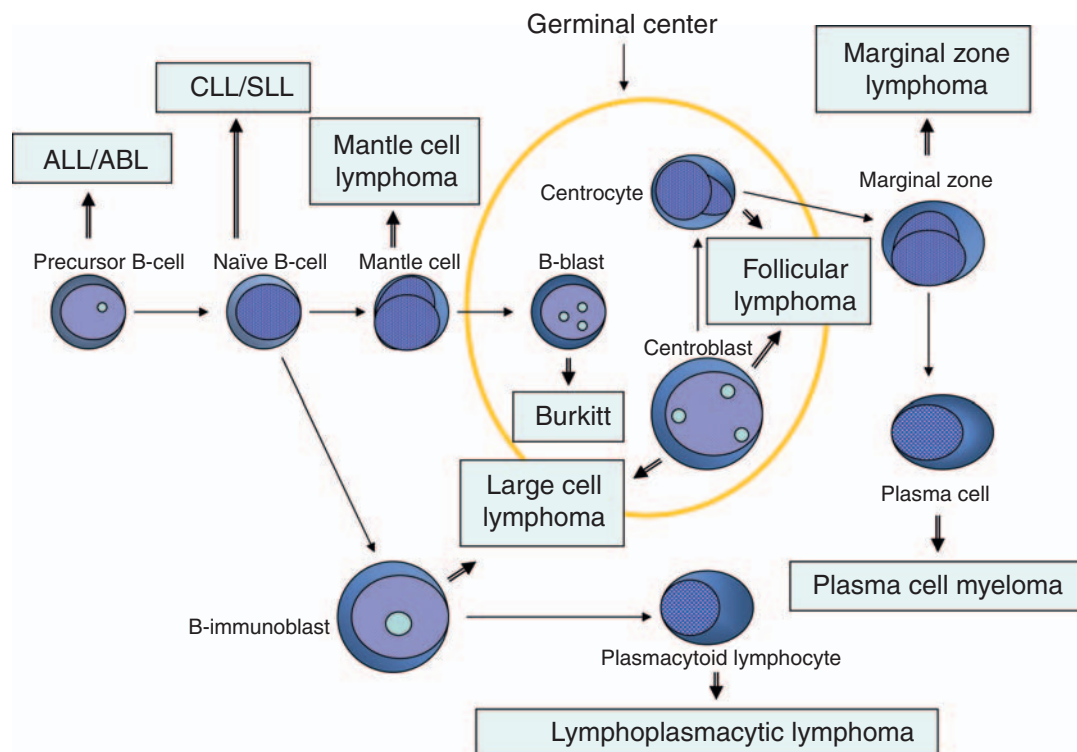


FIGURE 15.1 Scheme of B-cell differentiation and associated B-cell lymphomas. Adapted from Harris NL, Ref. [1].

14q32) as the first-line test, as this locus typically rearranges first in lymphocyte ontogeny and will thus be demonstrable in the highest proportion of neoplasms [3]. As noted earlier, the kappa light chain locus can then be examined subsequently for confirmation or further subclassification. Southern blot analysis was the first method devised for detection of immunoglobulin gene rearrangements, and in contrast to most other areas of the molecular pathology laboratory, it has yet to be entirely replaced by more modern PCR-based methods. The reason is that PCR cannot cover such a large region of gene comprehensively even with the use of multiplex primer sets typically directed at the V and J consensus sequences. Most laboratories now use a set of three “framework” primer pairs, designated FR1, FR2, and FR3 [4]. Depending on the subtype of B-cell tumor, the combined set will detect between 50% and 90% of clonal neoplasms. False-negative results can be due to the rearrangement breakpoints being out of coverage range of the primer sets used or because of interference with primer-target hybridization as a result of somatic hypermutation in the immunoglobulin genes themselves, another normal process for expanding antibody diversity. The latter phenomenon is especially frequent in follicular lymphomas (FLs), so that standard PCR methods have the lowest sensitivity (as low as 50%) for detecting clonality in these lesions [5]. In contrast, Southern blot methods using probes directed at the J_H region should detect >95% of clonal B-cell lesions assuming the specimen sent to the laboratory contains a sufficient proportion (at least 5–10%) of the malignant cells in question.

Gene rearrangement studies in B-cell lesions are performed for several purposes:

1. To distinguish malignant (clonal) lesions from reactive (nonclonal, polyclonal) lesions.
2. To determine likely cell of origin (B-cell versus T-cell) of the lesion.
3. To determine if a second (synchronous or metachronous) lesion represents the same or a different (new) clone.
4. To detect or monitor minimal residual disease (MRD) after initial therapy.

The first of these aims is accomplished by observing clonally rearranged band(s) separate from the persisting germline bands on Southern blot of extracted DNA hybridized with a probe directed at the J_H region. The rearranged band(s) can be anywhere in the lane, of either higher or lower molecular weight than the germline band(s); the location of the latter is determined by running a control sample (e.g. human placental DNA) that does not undergo gene rearrangement in the adjacent lane for each restriction endonuclease digest (Figure 15.2). Because of the chance that a single nongermline band could also result from a benign restriction fragment-length polymorphism (RFLP) present in this gene region, convention dictates that rearranged band(s) must be seen with at least two out of the three restriction enzymes used to call the specimen clonal [6]. One must also be careful to distinguish extra-germline artifacts

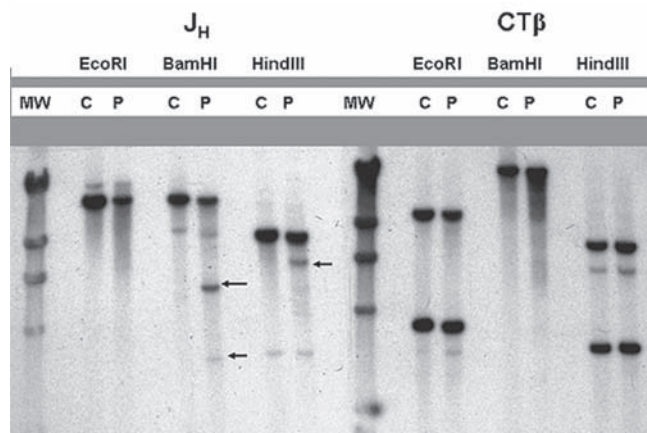


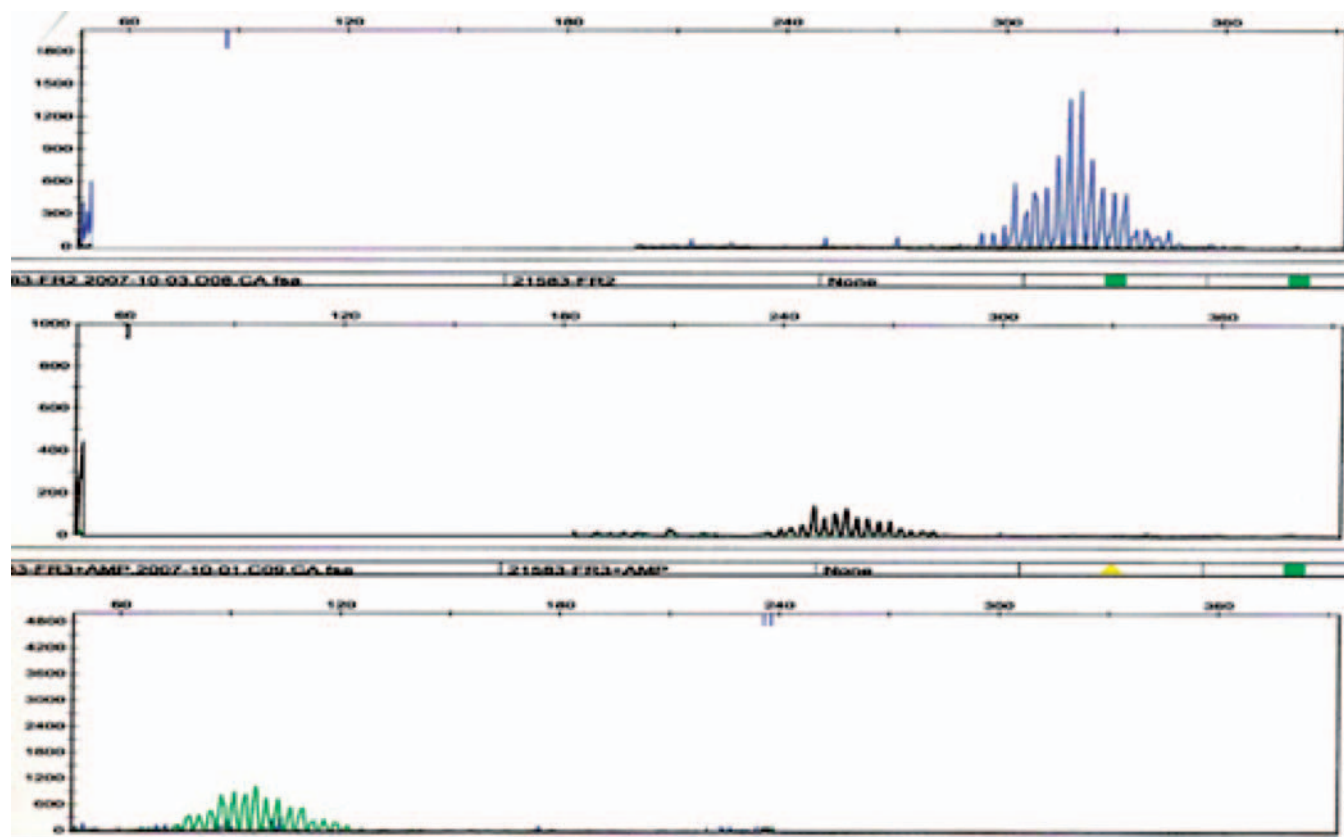
FIGURE 15.2 Southern blot analysis for clonality of immunoglobulin gene rearrangements in a B-cell lymphoma. Control (human placental) DNA (C) and patient (P) DNA were digested with three different restriction endonucleases (EcoRI, BamHI, and HindIII) and hybridized with either the J_H immunoglobulin heavy chain probe (left blot) or the CTB T-cell receptor probe (right blot) and subjected to autoradiography. Nongermline rearranged bands are seen in the patient's DNA with two of the restriction digests (arrows) hybridized with the J_H probe only; no rearranged bands are seen on the CTB blot, consistent with clonal B-cell origin.

due to partial restriction endonuclease digestion from true rearrangements. Alternatively, one can use a PCR assay comprised of the three framework primer sets. In this case, reactive lesions will demonstrate only a rounded and symmetrical polyclonal “smear” of amplicons representing the full range of benign rearrangements in the specimen, while a clonal lesion will demonstrate one or two sharp spikes representing an amplicon of discrete size derived from a clone of cells containing the same rearrangement. The clonal spikes may be freestanding or superimposed on the background polyclonal smear depending upon the percentage of clonal cells in the submitted specimen (Figure 15.3). Because the PCR method may miss a significant proportion of clonal B-cell lesions, for the reasons already discussed, a negative result does not exclude the possibility of a clonal B-cell population in the specimen. For this reason, in our laboratory we use the PCR method as an initial screen only: if it is “positive,” the case is signed out as such, but if it is “negative,” we reflex to Southern blot analysis for the definitive interpretation (except for paraffin-embedded tissue samples, which are not amenable to Southern blot and must be signed out based on the PCR results alone along with a prominent disclaimer about the potentially low sensitivity).

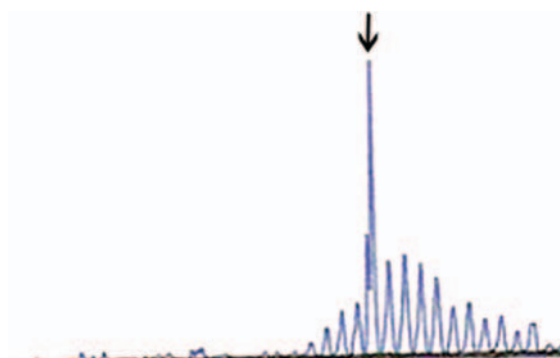
The second purpose of molecular and cytogenetic studies is to determine the cell of origin in a neoplastic process. While on the surface appearing relatively straightforward, here too, one must be mindful of exceptions to the rule. Though one may generally assume that lymphocytes with rearranged immunoglobulin heavy chain genes are B-cells, a small percentage of T-cell malignancies may rearrange their immunoglobulin genes, and vice versa [7]. In cases where doubt persists as to cell of origin, the analysis of J_K rearrangements may be informative, since these are more specific (though less sensitive) for B-cell malignancies.

The third purpose of these studies is the source classification of recurrent or multifocal lesions. As the gene rearrangement patterns observed on Southern blot or PCR are generally unique for a particular clone (out of the myriad possible rearrangements), they essentially establish a sort of DNA “fingerprint” for that clone. If analysis of a lymphoid lesion at another body site or a recurrence subsequent to treatment shows the identical pattern, it can be assumed to represent a metastasis or recurrence of the primary lesion [8]. Identity is assessed by looking for a clonal amplicon peak at the same molecular weight (length in base pairs) in PCR analysis (Figure 15.4) or a banding pattern (size and position) on Southern blot. This finding may have impact on the choice of therapy directed at the secondary lesion. Moreover, it is a conclusion not readily achieved by more traditional methods, since two lesions that appear to have similar histology or immunophenotype may in fact represent separate clones, whereas lesions with different histological and immunologic features could still represent evolution of the same clone.

The fourth purpose of gene rearrangement analysis, the detection of MRD, is actually more reliable in theory than in practice. Although one would like to be able to detect MRD as early and sensitively as possible after treatment in order to more proactively decide on subsequent management [9], the routine techniques described here for initial clonality determination at the time of diagnosis are not so ideal for sensitive detection of MRD after treatment. As noted, Southern blot analysis will detect a clone only if it represents 5–10% of the total cells in the specimen. And while one may always think of PCR as orders of magnitude more sensitive than Southern blot, that is not the case here. PCR is incredibly powerful at picking up a unique DNA sequence present in trace amount in a sample containing mostly nonhomologous sequences, as is the case when detecting the *BCR-ABL1* fusion gene in chronic myelogenous leukemia (see Chapter 9). The reason is that the primers are directed at the unique fusion sequence which should not be present in a normal cell population. But we have already stated that immunoglobulin gene rearrangement is a normal process that occurs in all lymphocytes, both benign and malignant, and the primers we use to assay clonality are based on consensus sequences which (barring somatic hypermutation) should not be significantly different between individuals, whether they have leukemia/lymphoma or not. Thus, the framework primers are competing for hybridization with both the clonal rearrangement and all the other benign rearrangements present in the sample. Since the thermodynamics of the hybridizations are essentially equivalent, there is no reason for the assay to preferentially pick out the clonal rearrangement, unless it is present in a predominant proportion of cells in the specimen, which will not be the case in MRD situations. In order to preferentially target the rearrangement in the malignant cells, primers must be designed that are patient specific and are based on DNA sequencing of the clone that was detected at the time of diagnosis. Then, typically using highly sensitive and quantitative real-time PCR approaches, the same clone can be identified after treatment even when the same is present in only trace amounts because the primers are (theoretically) not competing for all the other benign rearrangements in the



(a)



(b)

FIGURE 15.3 Examples of immunoglobulin gene clonality analysis by PCR. (a) Negative study showing only a polyclonal amplicon pattern with framework primers 1 (blue), 2 (black), and 3 (green). (b) Clonal peak (arrow) superimposed on a background polyclonal “smear” produced with the framework 1 primer set.

specimen [10]. Unfortunately, these methods are not generally available in routine clinical laboratories, so this application is generally relegated to special research situations.

The newest avenue of molecular testing of B-cell malignancies is gene profiling using gene expression-based microarrays. By detecting up- or downregulation of hundreds or thousands of genes in parallel, these lesions can be subclassified as to diagnosis and behavior in ways not possible by the more traditional methods [11, 12]. But, like the MRD approaches discussed earlier, these methods are at present restricted to the research setting. One possible

compromise for the clinical laboratory is to choose one or a few of the more dramatically correlated genes in the microarray, such as *ZAP-70*, and simply test for those to achieve an informative approach of the entire array [11, 13].

Mature B-cell neoplasms are divided into the following categories [1]:

Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL)

B-cell prolymphocytic leukemia (B-PLL)

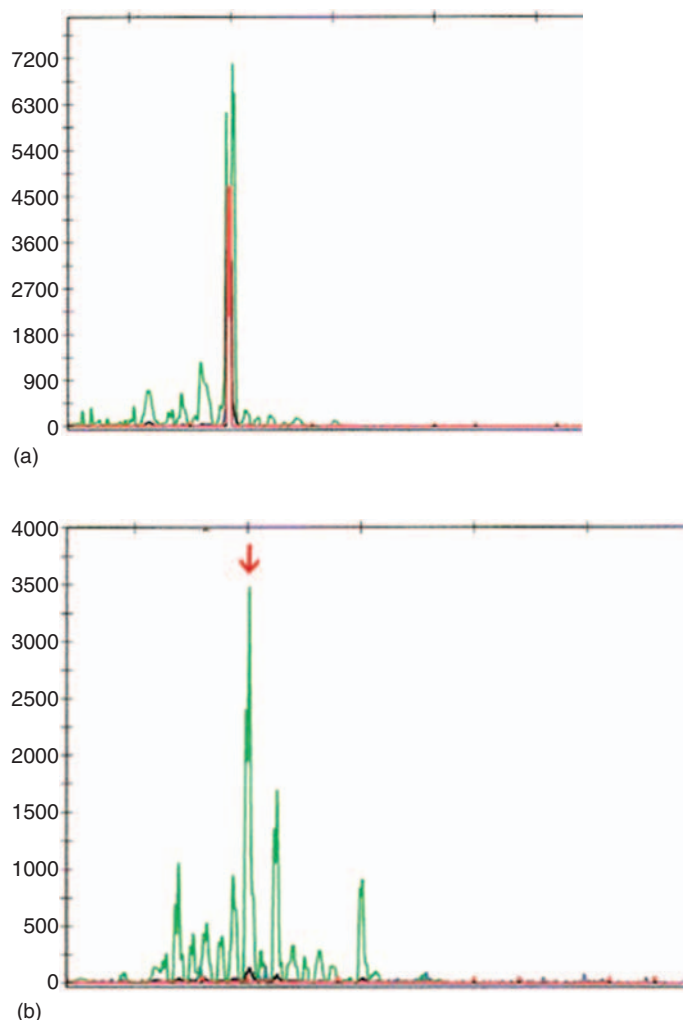


FIGURE 15.4 Immunoglobulin heavy chain clonality study in a patient with diffuse large B-cell lymphoma using PCR (framework 3 primer set results shown). (a) Clonal peak at 100bp at the time of initial diagnosis. (b) The results at start of relapse. An amplicon peak of the same size is seen, suggesting that this is a recurrence of the initial clone, though this time there is a more prominent background polyclonal population.

Lymphoplasmacytic lymphoma (LPL)
 Splenic marginal zone lymphoma (SMZL)
 Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue
 Nodal marginal zone B-cell lymphoma (nodal MZL)
 Hairy cell leukemia (HCL)
 Plasma cell disorders
 Follicular lymphoma (FL)
 Mantle cell lymphoma (MCL)
 Diffuse large B-cell lymphoma (DLBCL)
 Mediastinal (thymic) large B-cell lymphoma (MLBCL)

Intravascular large B-cell lymphoma (IVLBCL)

Primary effusion lymphoma (PEL)

Other variants of large B-cell lymphoma

Burkitt lymphoma/leukemia (BL)

All the above categories except for the plasma cell disorders are discussed in this chapter. Chapter 16 deals with the plasma cell disorders.

CHRONIC LYMPHOCYTIC LEUKEMIA/SMALL LYMPHOCYTIC LYMPHOMA

Chronic lymphocytic leukemia/small lymphocytic lymphoma is a lymphoproliferative disorder of small, mature B-lymphocytes primarily involving peripheral blood, bone marrow, and lymph nodes. The neoplastic cells are presumably derived from the naïve B-cells, characteristically coexpressing CD5 and CD23 (Figure 15.1). CLL/SLL is divided into two overlapping categories [1, 2, 14]:

1. CLL, primarily involving the bone marrow and the peripheral blood with or without lymph node involvement.
2. SLL, primarily involving lymph nodes with <30% bone marrow involvement and no peripheral blood lymphocytosis (non-leukemic).

Etiology and Pathogenesis

The etiology and pathogenesis of CLL/SLL are not known. A genetic background appears to play a role in the development of this disorder. Although CLL is considered the most common leukemia in the Western hemisphere, the incidence is very low in China and Japan. The incidence has remained low in the Japanese who have settled in Hawaii and the United States, suggesting a genetic role rather than environmental factors [15]. Certain genetic polymorphisms may predispose patients to CLL [16]. Familial risk of the development of CLL has been repeatedly discussed in the literature, including a series of 32 CLL families reported by the National Cancer Institute [17–21]. The familial susceptibility to CLL is observed in 5–10% of the patients [21]. No definitive environmental and occupational risk factors have been identified for CLL. Studies of the atomic bomb survivors during the period of 1950–1987 did not show a significant change in the incidence of CLL [22].

Pathology

Morphology

Peripheral blood and bone marrow lymphocytosis are morphologic hallmarks for the diagnosis of CLL. The majority of the lymphocytes are small and mature, appearing with a round nucleus, clumped chromatin, inconspicuous nucleoli, and scanty basophilic cytoplasm. Usually, a smaller

proportion of lymphoid cells (<10%) consist of prolymphocytes. Prolymphocytes are larger than lymphocytes with more abundant cytoplasm and a prominent nucleolus. About 30% of the patients with CLL show a white blood cell count of >100,000/ μ L.

The proposed criteria by the International Workshop on CLL (IWCLL) are as follows [23]:

1. A sustained peripheral blood lymphocyte count of >10,000/ μ L with most of the cells being mature-appearing lymphocytes.
2. A bone marrow aspirate showing >30% lymphocytes.
3. Peripheral blood lymphocytes that have a B-cell phenotype consistent with CLL (i.e. weak expression of surface Ig, CD5+, and rosette formation with mouse erythrocytes).

According to the IWCLL, the diagnosis of CLL is confirmed if criteria 1 and 2 or 1 and 3 are present. If the peripheral blood lymphocyte count is <10,000/ μ L, then both criteria 2 and 3 must be present in order to make a diagnosis of CLL.

The National Cancer Institute-sponsored Working Group (NCI-WG) has recommended the following criteria for the diagnosis of CLL [24, 25]:

1. Peripheral blood lymphocyte count of >5,000/ μ L with <55% of the cells being atypical (prolymphocytes). The cells should:
 - a. Express B-cell-specific differentiation antigens (CD19, CD20, CD23) and be positive for CD5, without other pan-T-cell markers.
 - b. Express restricted kappa or lambda light chain surface Ig, confirming monoclonality.
 - c. Express low density surface Ig.
2. Bone marrow aspirate showing >30% lymphocytes.

Patients with a blood count of \leq 5,000/ μ L may subsequently develop CLL. Follow-up white blood cell counts and flow cytometric studies are recommended for the adult patients with persistent lymphocytosis of 3,000–5,000/ μ L when immunophenotypic features are consistent with CLL. There is no evidence that early diagnosis of CLL in asymptomatic patients with mild lymphocytosis grants clinical benefits [26].

The blood smears show evidence of absolute lymphocytosis with >90% of lymphocytes consisting of small cells with round nucleus, coarse chromatin, indistinct or absent nucleoli, and scanty non-granular cytoplasm (Figure 15.5). Smudge and basket cells are frequently present, particularly in cases with high lymphocyte count (Figure 15.5b and c). These cells represent degenerated and destructed lymphocytes. Approximately 15% of the CLL patients show *atypical* morphologic features, such as increased proportion of prolymphocytes (PL; >10% but <55%), or presence of >15% lymphocytes with cleaved nuclei, or lymphoplasmacytic morphology (Figure 15.6). Cases with >10% and <55% prolymphocytes are referred to as CLL/PLL.

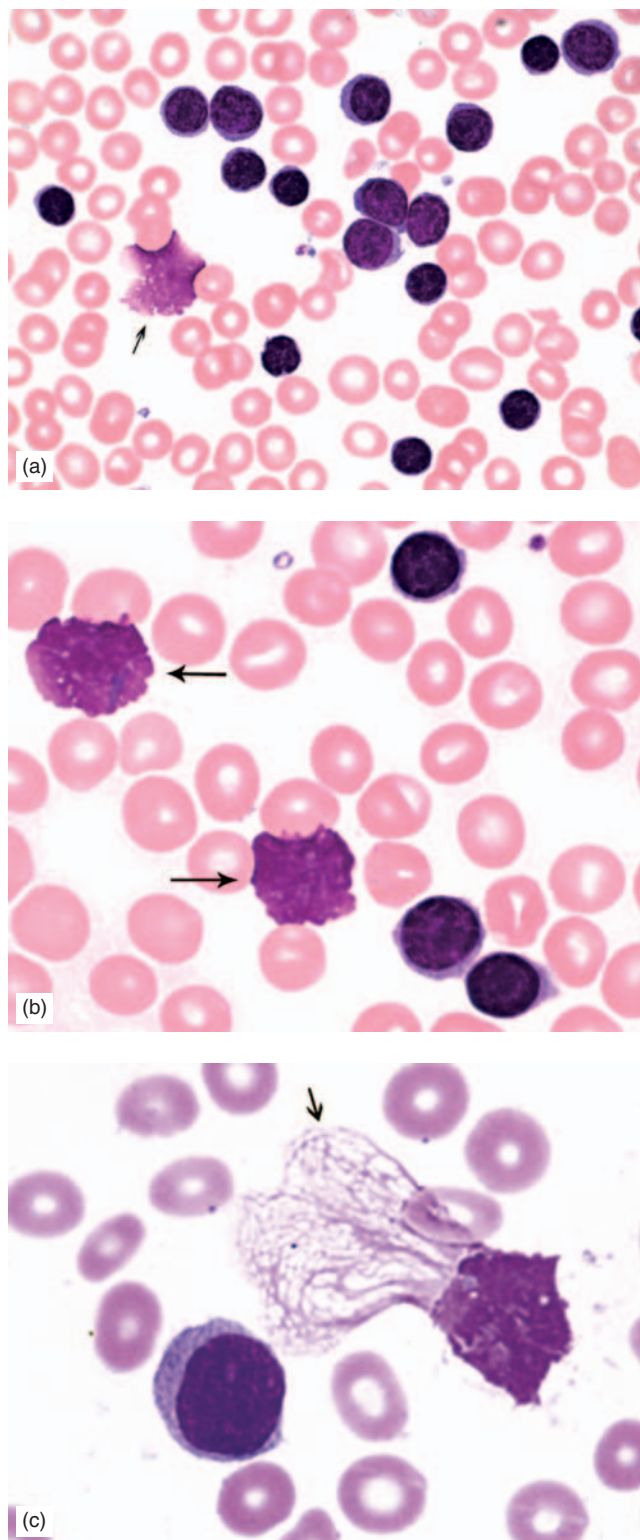


FIGURE 15.5 Blood smear of a patient with chronic lymphocytic leukemia demonstrating lymphocytosis with the presence of smudge cells (a and b, arrows) and a basket cell (c, arrow).

The pattern of involvement in the bone marrow biopsy sections is interstitial, nodular, diffuse, or a combination of these (Figures 15.7 and 15.8). The diffuse pattern is usually seen in the advanced stages of the disease. The lymphoid

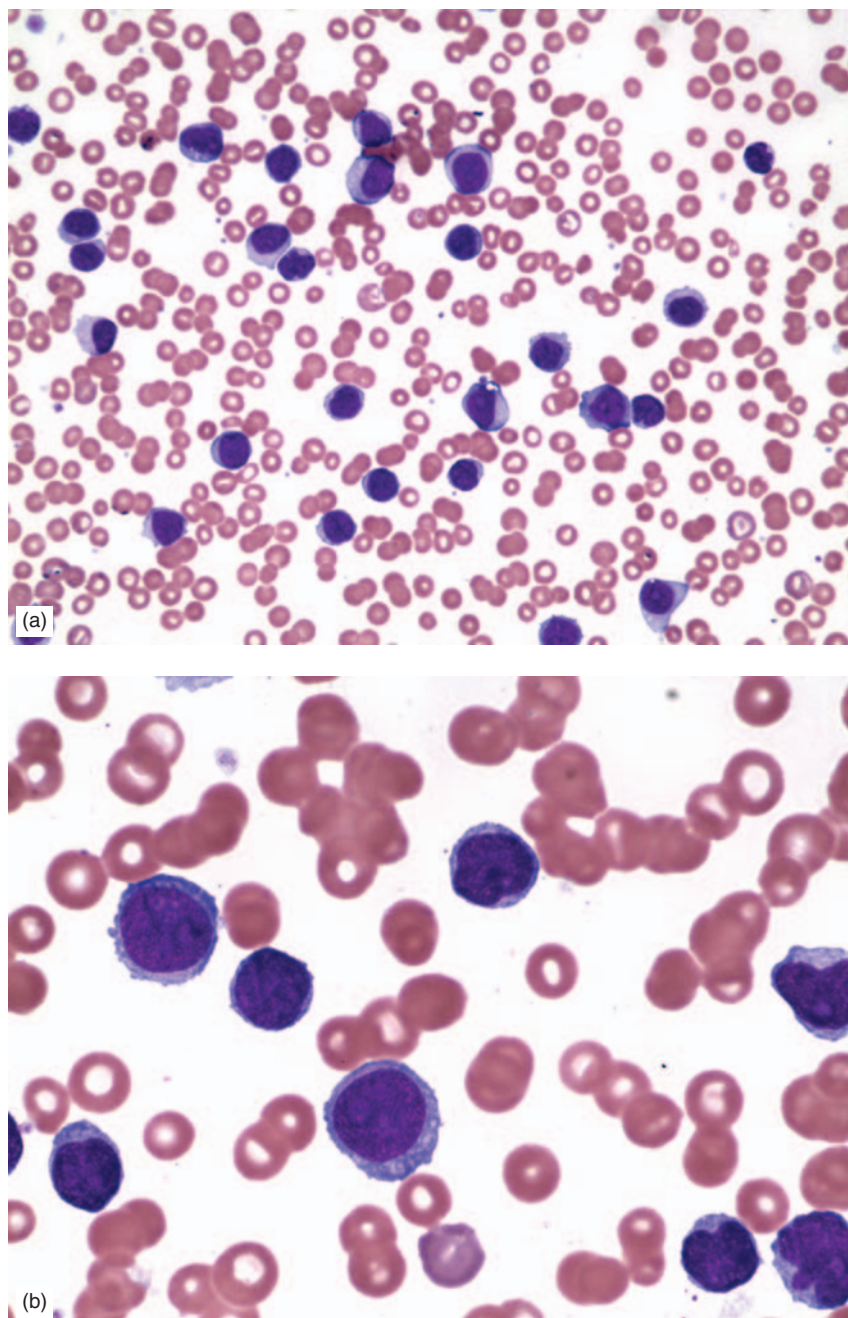


FIGURE 15.6 Blood smear of a patient demonstrating a mixture of lymphocytes and prolymphocytes: (a) low power and (b) high power views.

infiltrates consist of small, round lymphocytes with scattered prolymphocytes and larger cells called paraimmunoblasts. The lymphocyte count in the bone marrow smears in CLL is $>30\%$, whereas in SLL it is $\leq 30\%$.

The affected lymph nodes show architectural effacement with a diffuse infiltration by small lymphocytes. Characteristically, there are ill-defined paler areas with the predominance of prolymphocytes and paraimmunoblasts (Figure 15.9). These areas are called “proliferation centers” or “pseudofollicles”. Pseudofollicles are less frequently observed in the bone marrow and spleen. In some cases, there is a predominance of atypical lymphoid cells, such as lymphocytes with irregular nuclei or lymphoplasmacytic

cells. These cases may mimic mantle cell or LPLs. In the spleen, white pulp is the primary site of involvement, but the red pulp is also frequently involved.

Immunophenotype

The CLL cells express CD5, CD19, CD23, CD43, and HLA-DR and are weakly positive for surface Ig, CD20, and CD11c (Figures 15.10 and 15.11). The expression of CD22, CD79a, and FMC7 is weak or absent. The CLL cells are negative for CD10 and BCL-1, and in some cases may express CD25, CD38, ZAP-70, and CD79b [27–31].

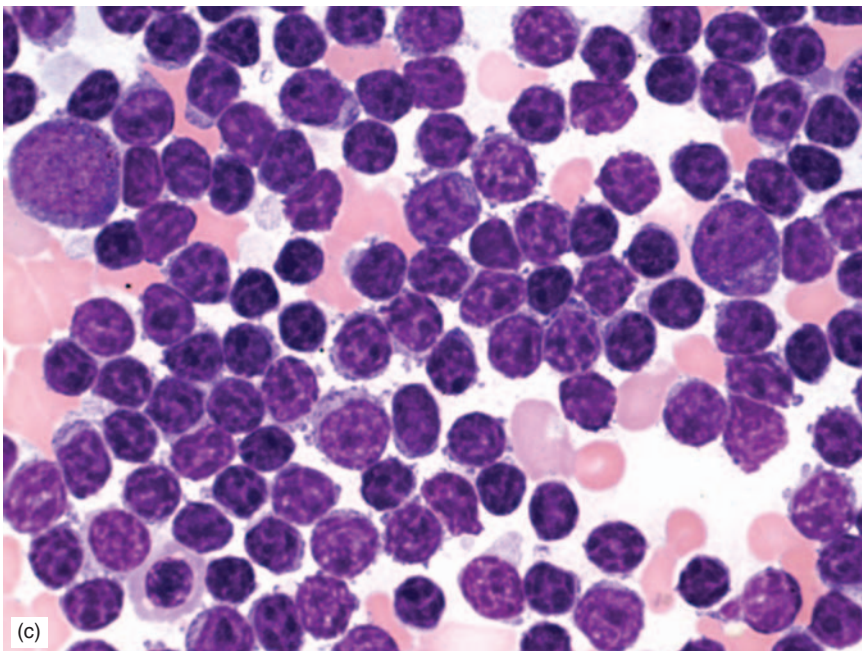
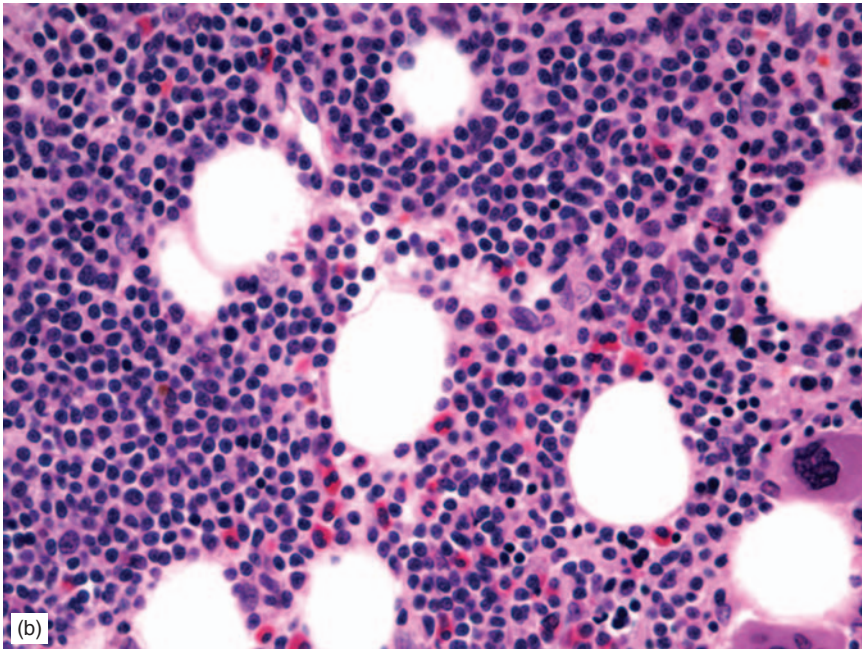
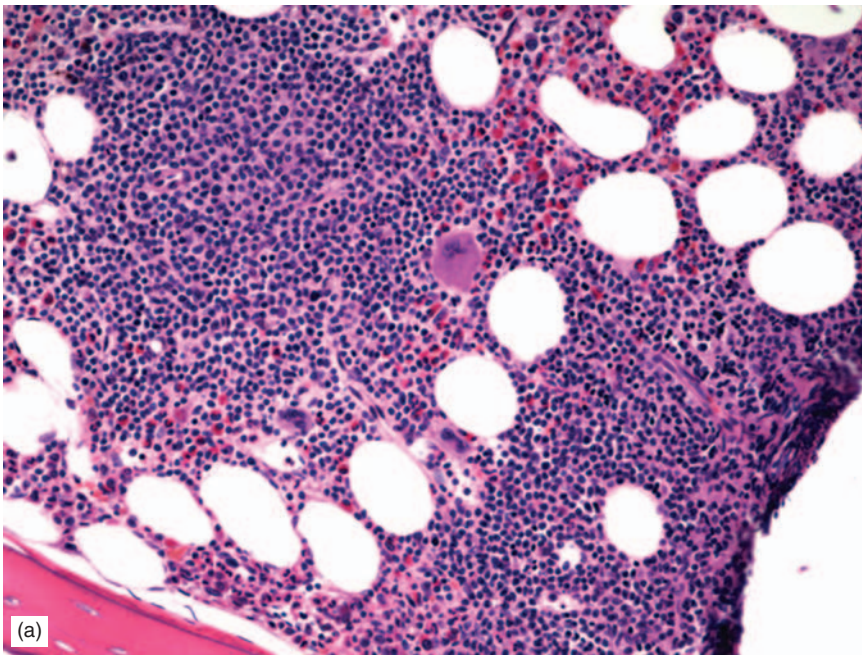


FIGURE 15.7 Bone marrow biopsy section (a and b) and bone marrow smear (c) showing involvement with chronic lymphocytic leukemia.

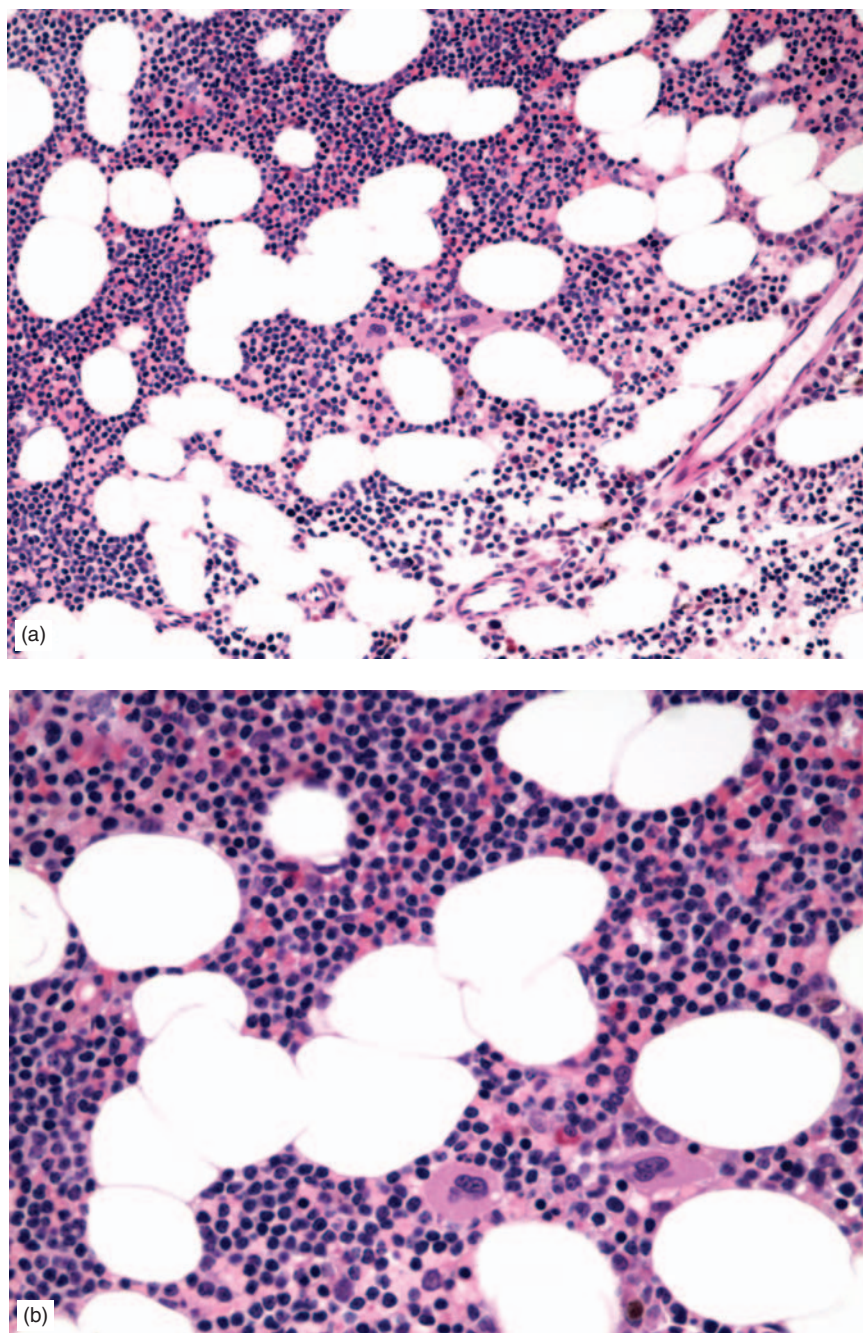


FIGURE 15.8 Bone marrow biopsy section demonstrating interstitial lymphoid infiltrate in a patient with chronic lymphocytic leukemia: (a) low power and (b) high power views.

A small proportion of CLL cases may totally lack the expression of CD5 or CD23 or partially express one or both of these markers. Also, some CLL cases may express strong FMC7 or CD20. These cases represent CLL with *atypical* immunophenotypic features.

The expression of CD38 and/or ZAP-70 is associated with an aggressive clinical course (see the following section) [32, 33]. CD38 is an ectoenzyme 45kD transmembrane glycoprotein which appears to contribute to proliferative potential of B-CLL cells, enhancing clinical aggressiveness of the disease [34]. ZAP-70 (zeta-chain associated protein of 70 kD) is an intracellular tyrosine kinase which is involved in TCR signaling and is highly expressed in B-CLL

cells which have unmutated *IgV_H* genes [35]. The expression of ZAP-70 is strong in normal T- and NK-cells and weak or negative in normal B-cells. Therefore, most flow cytometry laboratories use the patient's T- or NK-cells as internal positive controls, and the patient's normal B-cells (CD19+, CD5–) as internal negative control. ZAP-70 is considered positive when it is expressed by at least 20% of the CLL cells (Figure 15.12).

An immunophenotypic scoring system has been proposed for the diagnosis of CLL (Table 15.1). According to this scoring system, each of the following results is scored as for 1: weak expression of surface Ig, CD5 positivity, CD23 positivity, weak expression of CD22 or CD79a, and lack of

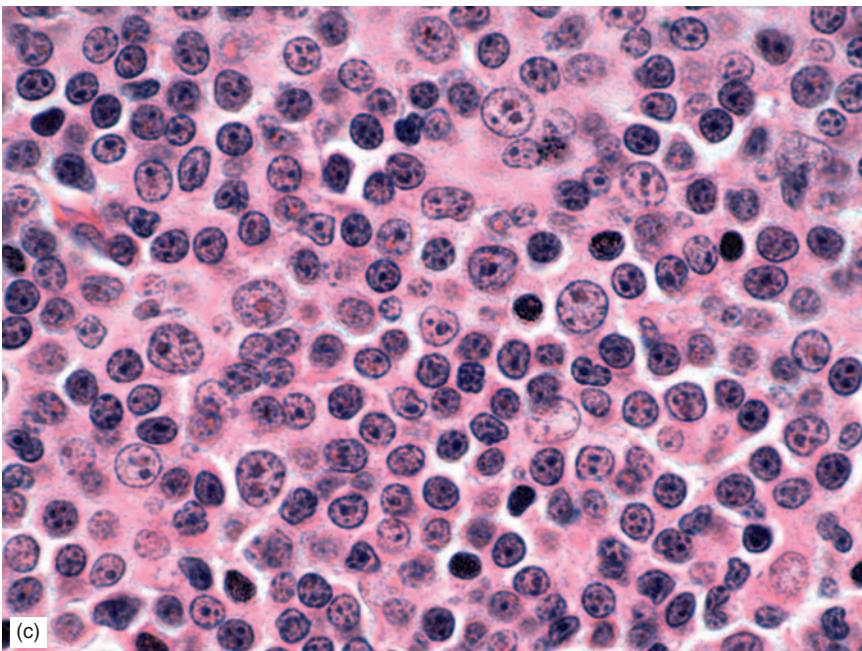
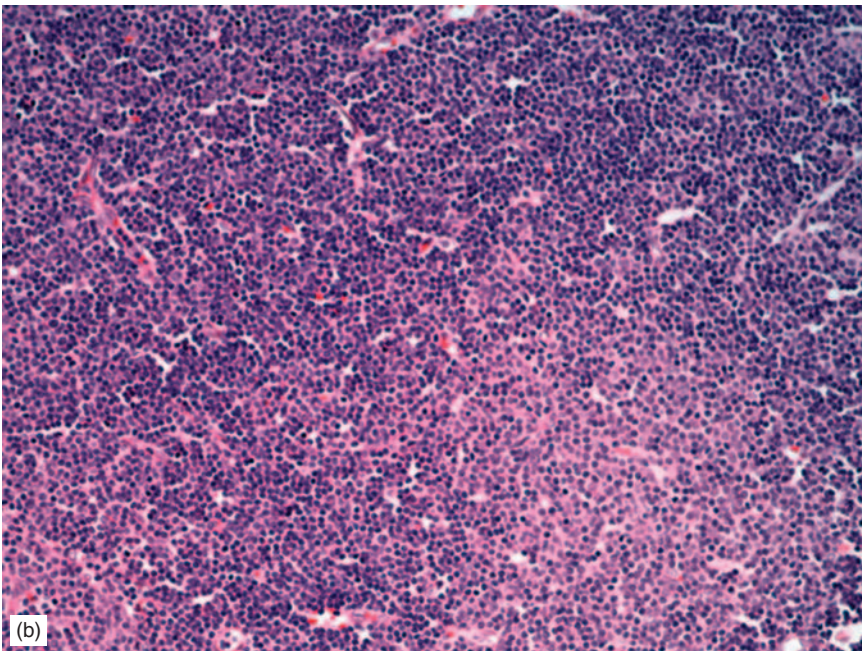
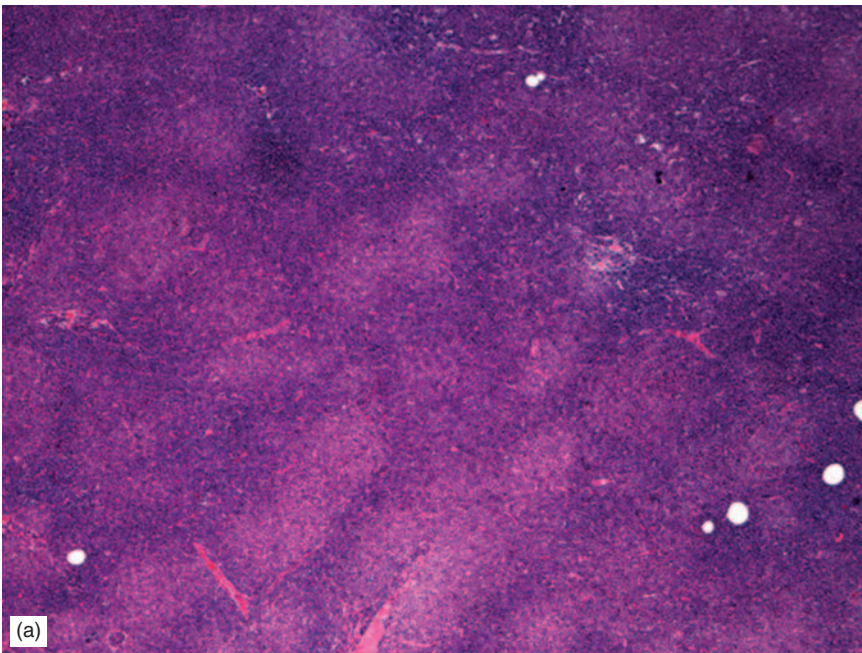


FIGURE 15.9 Lymph node section of a patient with chronic lymphocytic leukemia demonstrating proliferating centers or pseudofollicles (a and b, pale areas) consisting of a mixture of lymphocytes, prolymphocytes, and paraimmunoblasts (c).

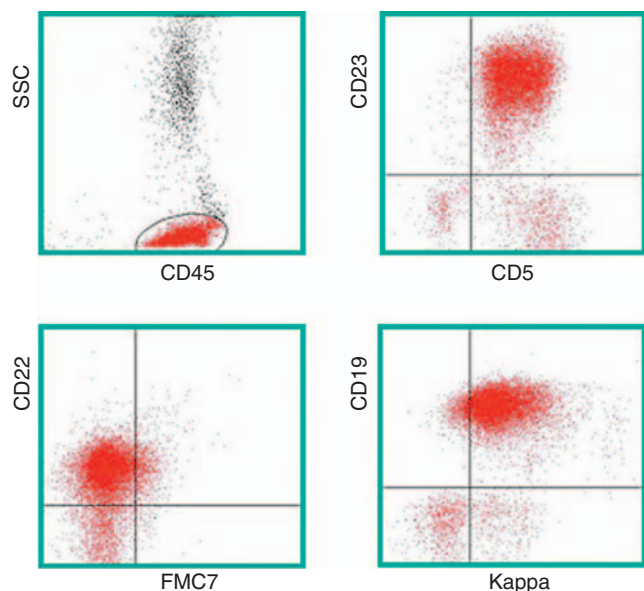


FIGURE 15.10 Flow cytometric analysis of a blood sample from a patient with CLL. The tumor cells are B-cells and characteristically coexpress CD5 and CD23.

expression of FMC7. Total scores of 4 or 5 are consistent with the diagnosis of CLL [36].

Aberrant expression of CD2, CD7, CD13, CD10, and CD34 has been reported in 10–30% of patients with CLL/SLL [37].

Cytogenetic and Molecular Studies

In CLL patients, karyotypic abnormalities tend to increase in frequency and number during the course of the disease. Chromosomal translocations, thought to occur mainly during the gene rearrangement process and common in other lymphoid malignancies, are rare in CLL. When translocations are found, they tend to result in a genetic loss rather than in the formation of a fusion gene or overexpression of an oncogene. These facts raise the pathogenetic possibility of missing tumor suppressor genes. Conventional cytogenetics detects structural chromosome abnormalities in about 40–50% of CLL patients [38–43].

Among patients with abnormal karyotypes, as many as 65% have one chromosome abnormality, 25% have two abnormalities, and the remainder have more complex abnormalities (6%) [4]. A 13q14 deletion is the most common finding (36–50% of the patients) [38, 39]; this deletion is believed to be a primary event in B-CLL, as it is present in a majority of the tumor cells and is frequently the sole abnormality (Figures 15.13 and 15.15). The second most common abnormality, and the most common abnormality to be detected by conventional cytogenetics, is trisomy 12 (11–21% of the patients) (Figures 15.14 and 15.15). Trisomy 12 usually displays an excess of large lymphocytes identifying the CLL mixed-cell-type variant of the FAB classification [42]. Trisomy 12 may be a secondary event in the course of CLL because it is typically identified in a minority of the tumor cells. Trisomy 12 is predominantly associated with unmutated V_H genes and seems to be associated

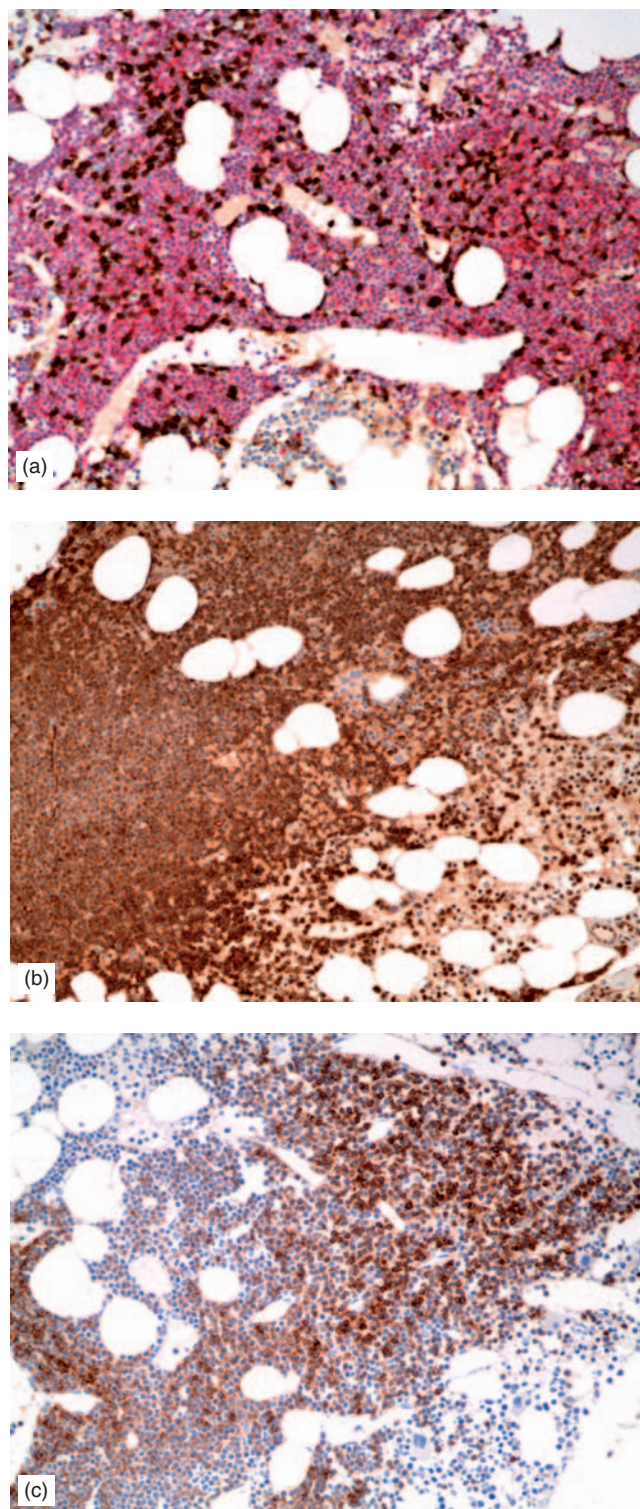


FIGURE 15.11 Immunohistochemical stains of a bone marrow biopsy section from a patient with CLL. (a) Dual staining demonstrating sheets of CD20+ cells (red) and scattered CD3+ cells (brown). The tumor cells also express CD5 (b) and CD23 (c).

with advanced or atypical cases of CLL. Less frequent primary aberrations in CLL include 14q32 rearrangements (up to 21%), 11q22.3 deletion (9–15%), and a 17p13 deletion (7–12%) (Figures 15.16 and 15.17) [41]. Other less

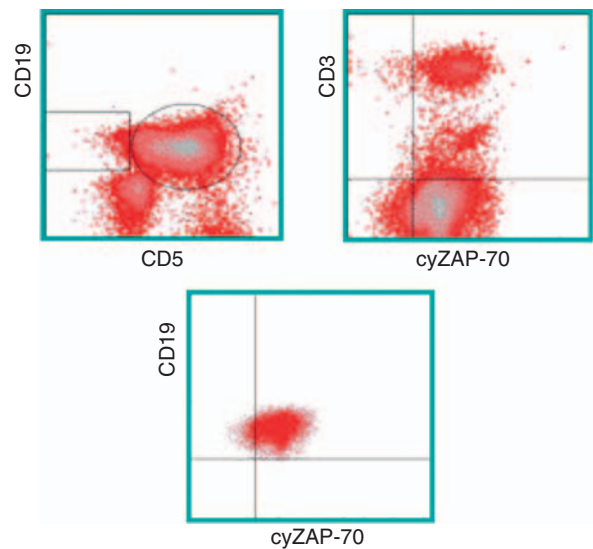


FIGURE 15.12 Flow cytometric analysis of ZAP-70. A large population of B-cells are CD5+, CD3–, and coexpress CD19 and ZAP-70.

TABLE 15.1 The immunophenotypic scoring system for the diagnosis of chronic lymphocytic leukemia/small lymphocytic lymphoma.*

Marker	Expression	Score**	Expression	Score
Surface Ig	Weak	1	Strong	0
CD5	Positive	1	Negative	0
CD23	Positive	1	Negative	0
CD22/CD79a	Weak or negative	1	Strong	0
FMC7	Negative	1	Positive	0

*Adapted from Refs [1, 26].

**Total scores in CLL/SLL are usually >3 and are often <3 in other lymphomas.

frequent chromosome abnormalities also occur (e.g. complex karyotypes).

A chromosome 6q deletion occurs in 7% of all CLL patients (as a primary event in 4%) and represents a cytogenetic and clinicobiological entity that exhibits a distinct phenotypic and hematologic profile. Patients with del(6q) usually present with a relatively high WBC count, classical immunophenotype, and CD38 positivity, which are associated with acceleration to the more aggressive prolymphocytic leukemia (PLL) [40]. Therefore, del(6q) patients require immediate therapy to achieve remission [42].

Chromosomal aberrations are not always detected in CLL patients' B-cells because it has been well noted that B-CLL cells are unresponsive to most lymphocyte mitogens and are extremely difficult to maintain in culture. For this reason, molecular cytogenetic techniques are more sensitive for the detection of clinically significant chromosome abnormalities than standard chromosome analysis. For example, fluorescence *in situ* hybridization (FISH) serves to unravel cryptic chromosomal aberrations that may not otherwise be detected due to the low mitotic index achieved in cultures obtained from the samples of most CLL patients even in the presence of B-cell mitogens [39, 41, 42, 44]. Additionally, when metaphases can be obtained, they are often so poor in quality that many aberrations escape detection. Therefore, FISH performed in conjunction with conventional cytogenetics is the methodology of choice for these disorders. All molecular cytogenetic techniques (i.e. FISH, CGH, and array CGH) have increased the detection rate of CLL to 80% [43, 44]. By using these techniques, as many as 65% of patients have one chromosome abnormality, 25% show two chromosome abnormalities, and the remaining 10% have more complex abnormalities. The recommended FISH panel for CLL detection consists of 11q22.3 (*ATM* gene), 13q14 (D13S319), *IGH* locus-specific probe (14q32), the centromere of chromosome 12 (D12Z3), and

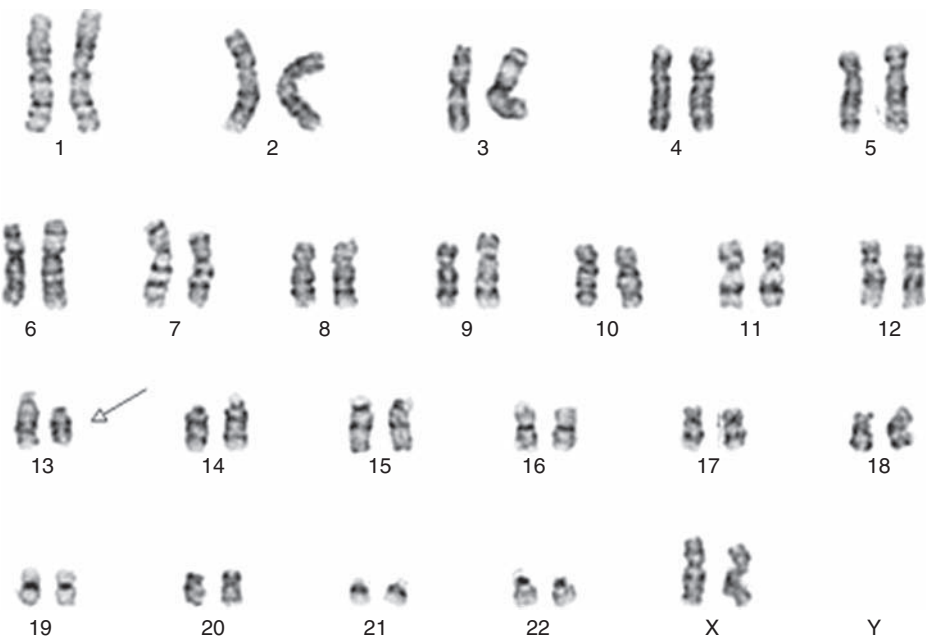


FIGURE 15.13 Bone marrow karyotype of a patient with CLL demonstrating 46,XX,del(13)(q12q14).



FIGURE 15.14 Bone marrow karyotype of a patient with CLL demonstrating 47, XX,+12

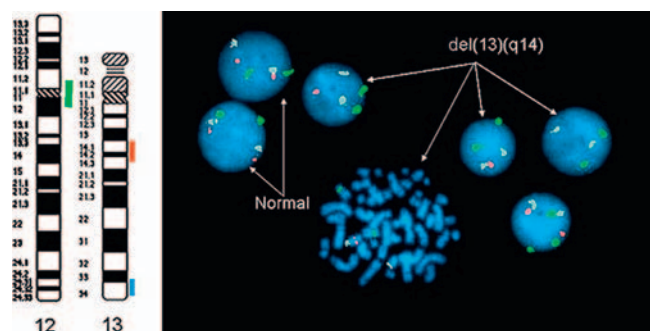


FIGURE 15.15 FISH analysis of cells from a patient with CLL showing two normal aqua (13q34), three green (Trisomy chromosome 12), and one red (deleted 13q14) signals.

17p13.1 (*p53* gene). In addition, there are recommendations to add 6q21 probe to the panel [41, 42].

The mutational status of the *IGHV* genes divides CLL into two major subtypes: mutated and unmutated [13, 45]. Approximately 45% of the CLL patients show no evidence of *V_H* gene mutation (unmutated). In general, these patients have an aggressive clinical course and advanced disease stage. The unmutated group shows a strong association with overexpression of ZAP-70 protein which could be detected by flow cytometry (see the following section).

Clinical Aspects

The most common type of leukemia in the Western countries is CLL/SLL accounting for about 40% of all leukemias in patients above 65 years of age. This disorder is extremely rare under the age of 30 years, but 20–30% of the patients are diagnosed under the age of 55 years [26, 46, 47]. The male:female ratio is about 2:1. Although the presence of familial aggregates of CLL has been well documented, the mode of inheritance is not known [20].

There is a sevenfold increase in the risk of CLL in first-degree relatives.

Approximately 25% of the patients are free of symptoms, and the CLL is an incidental finding during a routine blood examination. About 5–10% of the patients show systematic symptoms, such as weight loss, fever, night sweats, and/or extreme fatigue. Physical examination may reveal lymphadenopathy, splenomegaly, and hepatomegaly in approximately 85%, 50%, and 14% of the patients, respectively [47]. Autoimmune complications, primarily hemolytic anemia and thrombocytopenia, occur in up to 25% of the CLL/SLL patients [48].

The natural history of CLL/SLL is extremely variable with survival times ranging from 2 to 20 years [47]. Overall, the response rate to therapy and survival is better in women than in men [49]. Also, patients with atypical morphologic and/or immunophenotypic features tend to have a more aggressive clinical course. In general, presence and extent of lymphadenopathy, splenomegaly, hepatomegaly, anemia, and thrombocytopenia are the major clinical parameters that correlate with prognosis. Two major clinical staging systems have been developed by Rai *et al.* and Binet *et al.* [50, 51]. The original staging system proposed by Rai consisted of six stages from 0 to 5. This staging system was later (1987) modified and simplified to three major groups of low risk, intermediate risk, and high risk (Table 15.2) [26, 47]. In the Binet staging system, there are five designated sites of involvement demonstrated by cervical, axillary, and inguinal lymphadenopathies (unilateral or bilateral), splenomegaly, and hepatomegaly. Anemia and/or thrombocytopenia represent an advanced stage (Table 15.3).

Several biomarkers are indicative of aggressive clinical course in CLL/SLL. These include expression of CD38 and ZAP-70, unmutated *IGHV* (*V_H*) genes, del(11q22.3), and del(17p13.1). Several studies indicate a significantly less favorable median survival for patients with unmutated *V_H* and CD38-positive CLL cells than for patients with mutated *V_H* and CD38-negative tumor

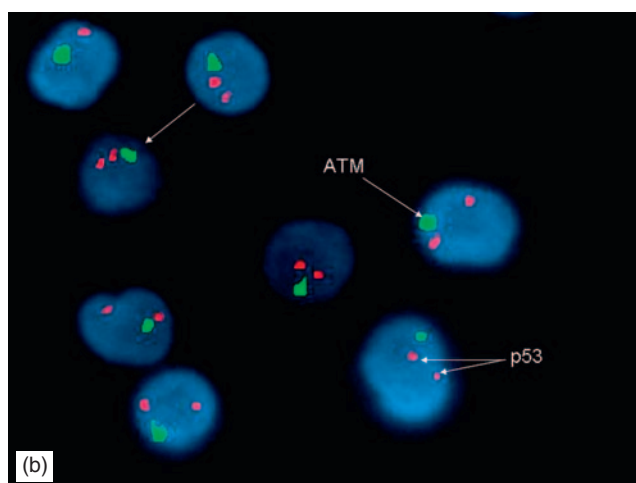
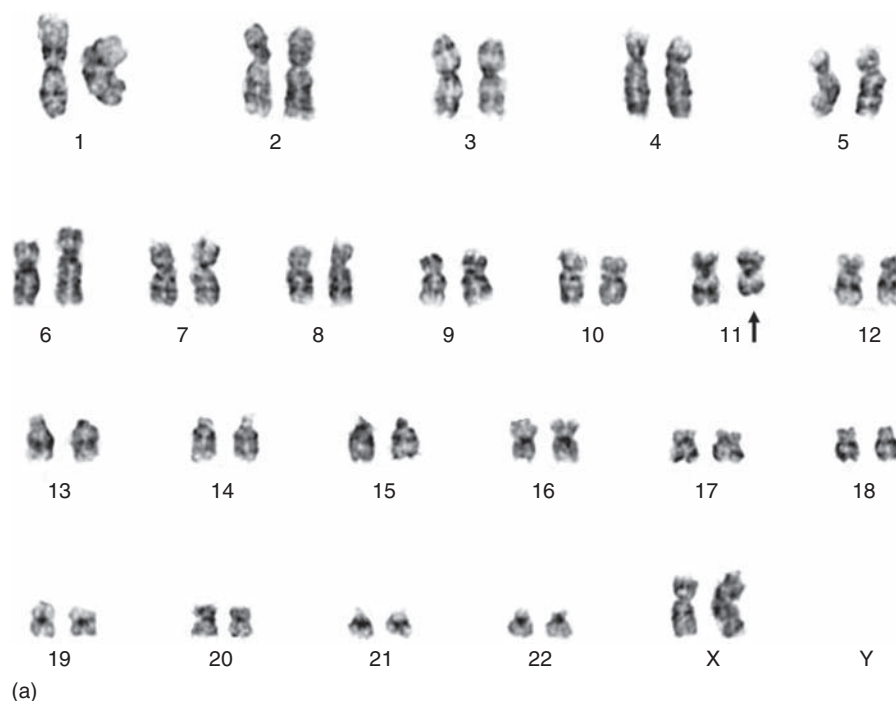


FIGURE 15.16 (a) Bone marrow karyotype of a patient with CLL with a 46,XX,del(11)(q22.1) and (b) FISH analysis with the ATM probe (green signals) and TP53 (red signals) on the same sample reveals a deletion of the 11q22 locus.

cells [52–55]. There is a strong correlation between unmutated V_H and overexpression of ZAP-70 in CLL cells. Also, there is an association between ZAP-70 expression and del(11q22.3) [56].

Cytogenetics is also helpful in predicting the course of CLL [42–44]. In fact, chromosomal abnormalities are independent predictors of disease regression and survival. Patients with diploid karyotypes or a 13q deletion as a sole abnormality have the best prognosis and a benign clinical course (median survival 79–133 months) [39, 40, 44]. The presence of the del(6q) or trisomy 12 usually has an adverse effect on patient's survival and results in intermediate prognosis (median survival 33–114 months) [39]. Patients

with 11q22–23 (median survival 13–79 months), 17p13 deletion (median survival 9–32 months), and complex karyotypes have the worst prognosis [13, 40–51, 57].

Although data related to chromosomal abnormalities is important in determining a diagnosis and prognosis for CLL patients, it is also useful for additional applications, such as detecting MRD, and possibly indicating potential target sites for therapeutic interventions. Jahrsdorfer *et al.* showed that cytogenetic status correlates with the biological behavior of B-CLL *in vitro* [38]. Poor prognostic cytogenetics was associated with more rapid spontaneous apoptosis *in vitro*, lower immunogenicity, and higher lactate dehydrogenase (LDH). Good prognostic cytogenetics was associated

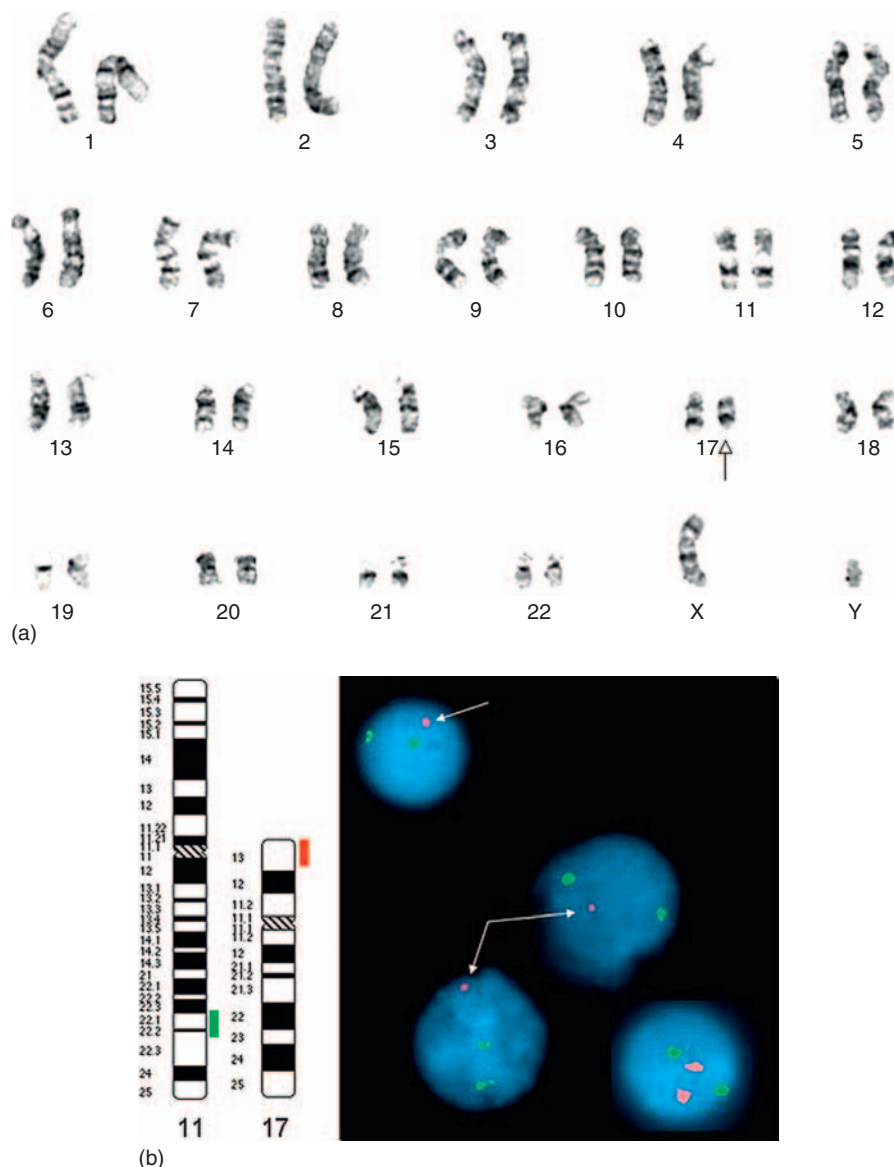


FIGURE 15.17 Bone marrow karyotype (a) and FISH analysis (b) demonstrating del(17)(p11.2).

with less spontaneous apoptosis, higher *BCL-2* levels, stronger immunogenicity, and lower levels of LDH [38].

A number of serologic parameters such as elevated levels of β 2-microglobulin soluble CD23 are reported in association with an aggressive clinical course in CLL patients. The prognostic factors in CLL/SLL are summarized in Table 15.3.

Several treatment modalities are available for patients with CLL/SLL [26, 58]. The therapeutic approaches are mainly based on the patient age and physical status, stage of the disease and cytogenetic findings. A “watch and wait” approach may be chosen for patients in early stage of disease or low-risk category, whereas patients with advanced or high-risk disease usually receive treatment. Alkylating agents, purine analogs, and monoclonal antibodies (such as rituximab) are frequently utilized in the therapeutic protocols.

Transformation of CLL to a More Aggressive Disease (Richter Syndrome)

Development of a high-grade non-Hodgkin lymphoma in patients with CLL was first described by Richter in 1928 (Figure 15.18). The term “Richter syndrome” was later referred to the transformation of CLL to a wide variety of more aggressive lymphoid malignancies, such as large cell lymphoma, prolymphocytic leukemia, lymphoblastic lymphoma, Hodgkin lymphoma, and plasma cell myeloma [59–62]. The incidence of Richter syndrome in CLL is about 5–10% with prolymphocytic leukemia being the most frequent type of transformation. The transformed cells may arise from the original CLL clone or may represent a new neoplastic clone, and the sequence-specific quantitative PCR methods discussed earlier can be used to distinguish between these two possibilities. The exact mechanism(s)

TABLE 15.2 The Rai and Binet staging systems in chronic lymphocytic leukemia/small lymphocytic lymphoma.*

Staging system	Features	Frequency (%)
<i>Modified Rai (1987)</i>		
Low risk (stage 0)	Lymphocytosis only	30
Intermediate risk (stages I and II)	Lymphadenopathy and/or hepatosplenomegaly	60
High risk (stages III and IV)	Hemoglobin <11 g/dL and/or platelet count <100,000/ μ L	10
<i>Binet (1981)</i>		
Stage A	<3 lymphoid areas**	60
Stage B	>3 lymphoid areas	30
Stage C	Hemoglobin <10 g/dL and/or platelet counts <100,000/ μ L	10

*Adapted from Refs [26, 47].

**Lymphoid areas are designated: Unilateral or bilateral cervical lymphadenopathy, and inguinal lymph; unilateral or bilateral axillary lymphadenopathy; unilateral or bilateral inguinal lymphadenopathy; splenomegaly; hepatomegaly.

TABLE 15.3 Prognostic factors in chronic lymphocytic leukemia/small lymphocytic lymphoma.*

Factor	Low risk	High risk
Gender	Female	Male
Clinical stage		
Binet	A	C
Rai	0	III and IV
Lymphocyte morphology	Typical	Atypical
Bone marrow involvement	Non-diffuse	Diffuse
Elevated levels of serum β 2-macroglobulin and CD23	Not present	Present
CD38 expression	Negative	Positive
ZAP-70	Negative	Positive
<i>IgVH</i> gene status	Mutated	Unmutated
Cytogenetics	Normal or del(13q14)	del(17p13) or del(11)(q22)

*Adapted from Ref. [26].

of Richter transformation is not well understood. Multiple genetic abnormalities such as p53 mutation, deletion of retinoblastoma gene (13q14), increased copy number of *C-MYC* and decreased expression of *c-MYB* gene have been described [59]. Trisomy 12 and 11q aberrations are more frequent in Richter syndrome than in the overall CLL population [59, 60].

In most instances, Richter transformation is associated with the development of systemic symptoms, such as fever,

weight loss, and night sweats, and/or a rapid organomegaly, such as increased lymphadenopathy, splenomegaly, and/or hepatomegaly. The site of transformation is usually lymph node or bone marrow, and occasionally extranodal/extramedullary sites, such as the skin, gastrointestinal tract, and central nervous system. Richter transformation is associated with a rapid clinical deterioration and a low response rate to therapeutic strategies. The median survival duration has been reported between 5 and 8 months [59].

Differential Diagnosis

The differential diagnosis comprises conditions associated with absolute peripheral blood lymphocytosis, increased proportion of CD5+ B-cells, and the presence of a monoclonal population of B-cells.

Chronic polyclonal B-cell lymphocytosis is a rare reactive lymphoproliferative disorder often observed in middle-aged women with a history of heavy smoking. The absolute blood lymphocyte count ranges from 4,000 to 20,000/ μ L with the presence of activated and binucleated lymphocytes (see Chapter 19). The majority of the lymphocytes are B-cells. These cells, unlike CLL cells, are polyclonal and lack CD5 expression.

CD5+ B-lymphocytes comprise a subset of the B-cells in normal individuals. In most studies, they account for up to 25% of the B-cells, though there are reports claiming that up to 47% of the normal B-cells may coexpress CD5 [63, 64]. A more recent study by Gupta and associates reported a mean percentage of about 12% CD5+ B-cells in the peripheral blood and bone marrow of the normal individuals. The expression of CD5 is dim on the normal B-cells and brighter on the CLL cells [65]. The recommended cutoff point for the detection of minimal residual CLL in treated patients is $\geq 25\%$ CD5+ B-cells in the peripheral blood or in the bone marrow samples.

Monoclonal B-cell expansion in the elderly is a condition reported in about 3.5% of healthy individuals above 65 years of age with no evidence of absolute lymphocytosis or history of lymphoid malignancy. The immunophenotypic features of these monoclonal B-cells can be divided into two major groups: CLL-like and non-CLL-like.

The CLL-like phenotype is characterized by CD19+, CD23+, CD5+, FMC7–, and CD10–. The CD20 expression may be dim (typical) or strong (atypical). This phenotype has been observed in up to 13.5% of healthy relatives of patients with CLL [65, 66].

The non-CLL-like phenotype is characterized by CD19+, CD20+, CD23–, CD5–, FMC7+, and CD10–. This phenotype has been referred to as monoclonal B-lymphocytosis of undetermined significance (MLUS) by some investigators [67].

Mantle cell lymphoma and LPL share overlapping morphologic features with CLL. The neoplastic cells in MCL express BCL-1 protein and are usually negative for CD23. The cytogenetic hallmark for MCL is t(11;14). The immunophenotype of LPL is characterized by the lack of expression of CD5, CD10, and CD23 and the expression of surface and cytoplasmic IgM. A significant proportion

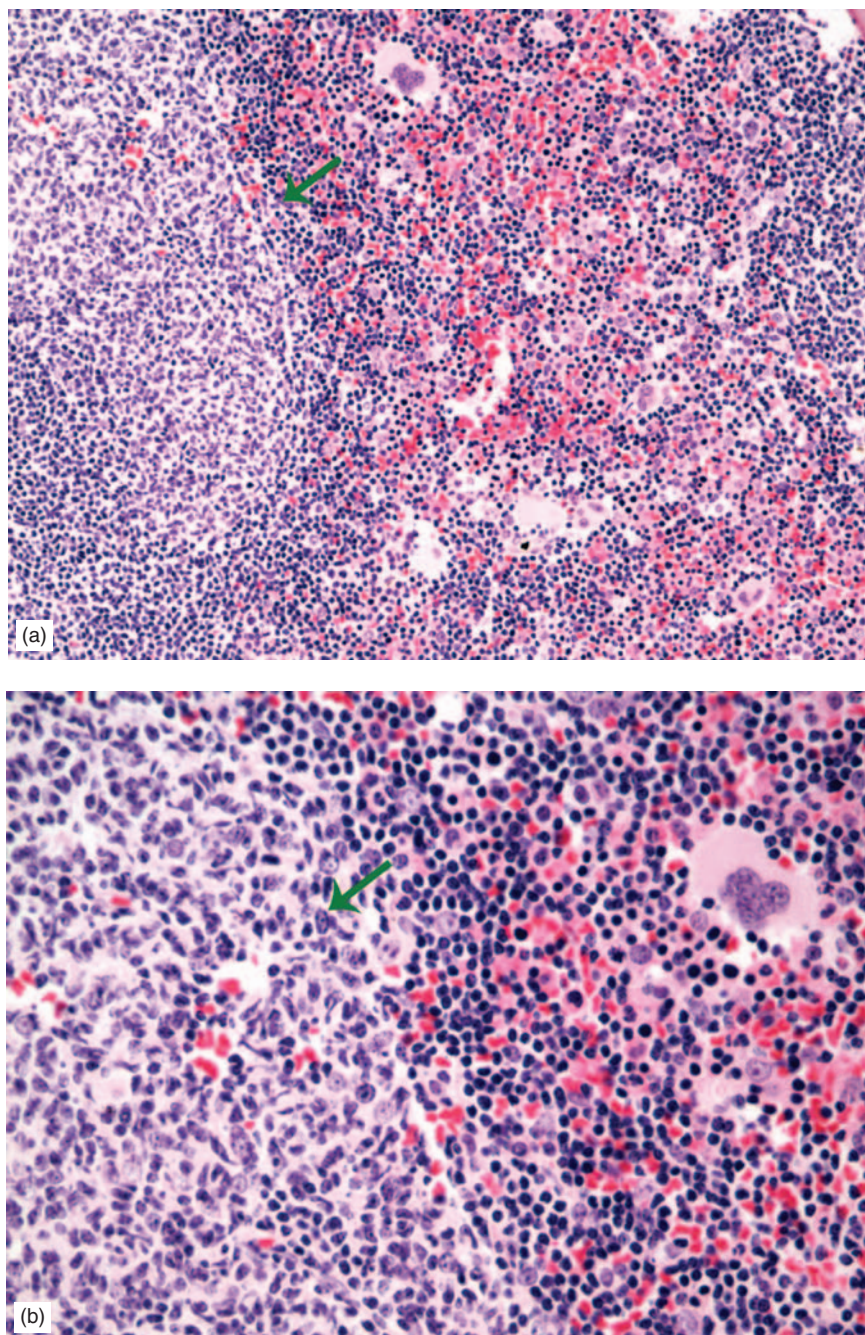


FIGURE 15.18 Richter syndrome. Bone marrow biopsy section from a patient with CLL demonstrating a focal area (arrow) of transformation to large cell lymphoma; (a) low power and (b) high power views.

of patients with LPL show $\text{del}(6)(\text{q}21 \rightarrow \text{q}23)$ or $\text{t}(9;14)$ (discussed later). A comparison of immunophenotypic and cytogenetic features in CLL/SLL, LPL, and MCL is presented in Table 15.4.

B-CELL PROLYMPHOCYTIC LEUKEMIA

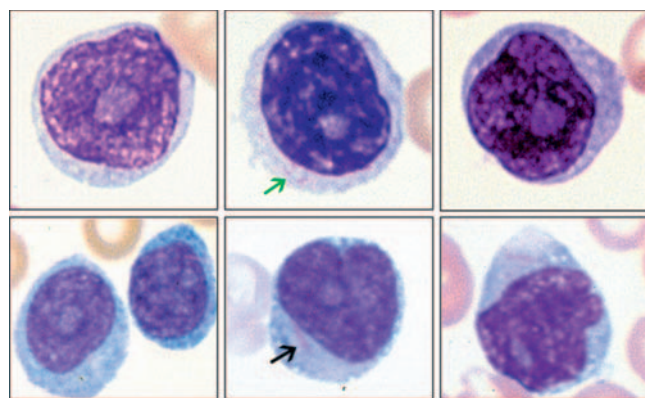
B-cell prolymphocytic leukemia (B-PLL) is a rare lymphoproliferative disorder characterized by the clonal

proliferation of prolymphocytes primarily involving blood, bone marrow, and spleen [1, 68–70]. Prolymphocytes are medium-sized cells with variable amounts of light basophilic cytoplasm, usually round nucleus, moderately condensed chromatin, and a prominent nucleolus (Figure 15.19). In prolymphocytic leukemia, prolymphocytes account for $>55\%$ of the lymphoid cells. B-cell leukemias with prolymphocyte-like features are divided into three groups:

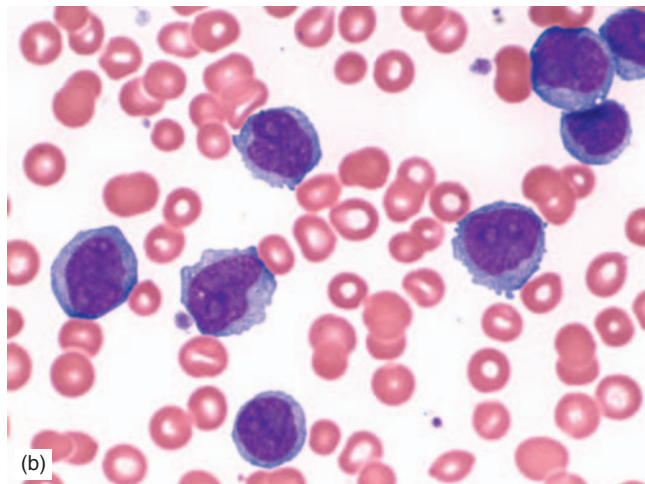
1. *De novo* B-PLL.
2. Prolymphocytic leukemia evolved from the transformation of CLL [71].

TABLE 15.4 Comparison of immunophenotypic and cytogenetic features in chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), lymphoplasmacytic lymphoma (LPL), and mantle cell lymphoma (MCL).

Immunophenotype	CLL/SLL	LPL	MCL
CD5	+	—	+
CD10	—	—	—
CD19	+	+	+
CD20	Dim	+	+
CD22	Dim	+	+
CD23	+	+/- (dim)	—
CD79a	Dim	+	+
FMC7	—	+	+
BCL-1	—	—	+
Cytogenetics	del(17)(p13) del(11)(q22) Trisomy12 del(13)(q14)	t(9;14)	t(11;14)



(a)



(b)

FIGURE 15.19 Polymphocytes in the blood smear of patients with polymphocytic leukemia (a and b). Some polymphocytes may show cytoplasmic granules (a, green arrow) or inclusions (a, black arrow).**3. Leukemic phase of MCL with polymphocytic morphology and evidence of t(11;14) [70, 71].**

In this section the *de novo* B-PLL is discussed.

Etiology and Pathogenesis

The etiology and pathogenesis of B-PLL are not known.

Pathology**Morphology**

Peripheral blood, bone marrow, and spleen are the major sites of involvement. There is a marked peripheral blood lymphocytosis (usually >100,000/mL) with the presence of >55% polymphocytes. Polymphocytes comprise >55% of the lymphoid cells in the peripheral blood. Polymphocytes are larger and contain more cytoplasm than CLL cells (Figure 15.19). In most cases, they display a round nucleus with moderately condensed chromatin and prominent nucleolus (Figure 15.20). In rare cases, however, the nucleus is irregular or indented, and there are more than one prominent nucleoli. Occasionally, a small proportion of polymphocytes may show cytoplasmic granules or inclusions [74]. The bone marrow biopsy sections often show a diffuse infiltration by the neoplastic polymphocytes.

Splenic involvement is a frequent finding. Both white and red pulps are extensively infiltrated by polymphocytes. The white pulp is markedly expanded and the red pulp is diffusely or patchily involved, often creating a mixture of diffuse and nodular patterns. Some of the extended white pulp nodule may show smaller lymphoid cells in the center surrounded by larger cells in the periphery [1].

Immunophenotype

B-cell polymphocytic leukemia cells express surface Ig (IgM with light chain restriction) and B-cell-associated CD molecules, such as CD19, CD20, CD22, CD79a, and FMC7. They are negative for CD10 and CD23. CD5 is positive in approximately 30% of the cases [1], and over 50% of the cases express CD38 and/or ZAP-70 [68].

Cytogenetic and Molecular Studies

The chromosomal aberration of t(11;14)(q13;q32) frequently reported in leukemias with polymphocytic morphology now, in most instances, is considered to represent the leukemic phase of a subtype of MCL [68, 72, 73]. Trisomy 12, del(11)(q22), and del(13)(q14) that are typically reported in CLL/SLL have also been reported in a number of B-PLL cases. However, some of these cases may represent the PLL transformation of CLL/SLL [71]. Several studies report karyotypic abnormalities involving 17p13 and 8q24, the sites of the *p53* and *c-MYC* genes, respectively (Figure 15.21). Loss of heterozygosity at 17p13 has been reported in 53% of the B-PLL patients [75, 76]. In one study, a complex karyotype was reported by demonstrating der(14)t(14;17) and t(2;8), involving both *p53* and *c-MYC* genes [75, 76].

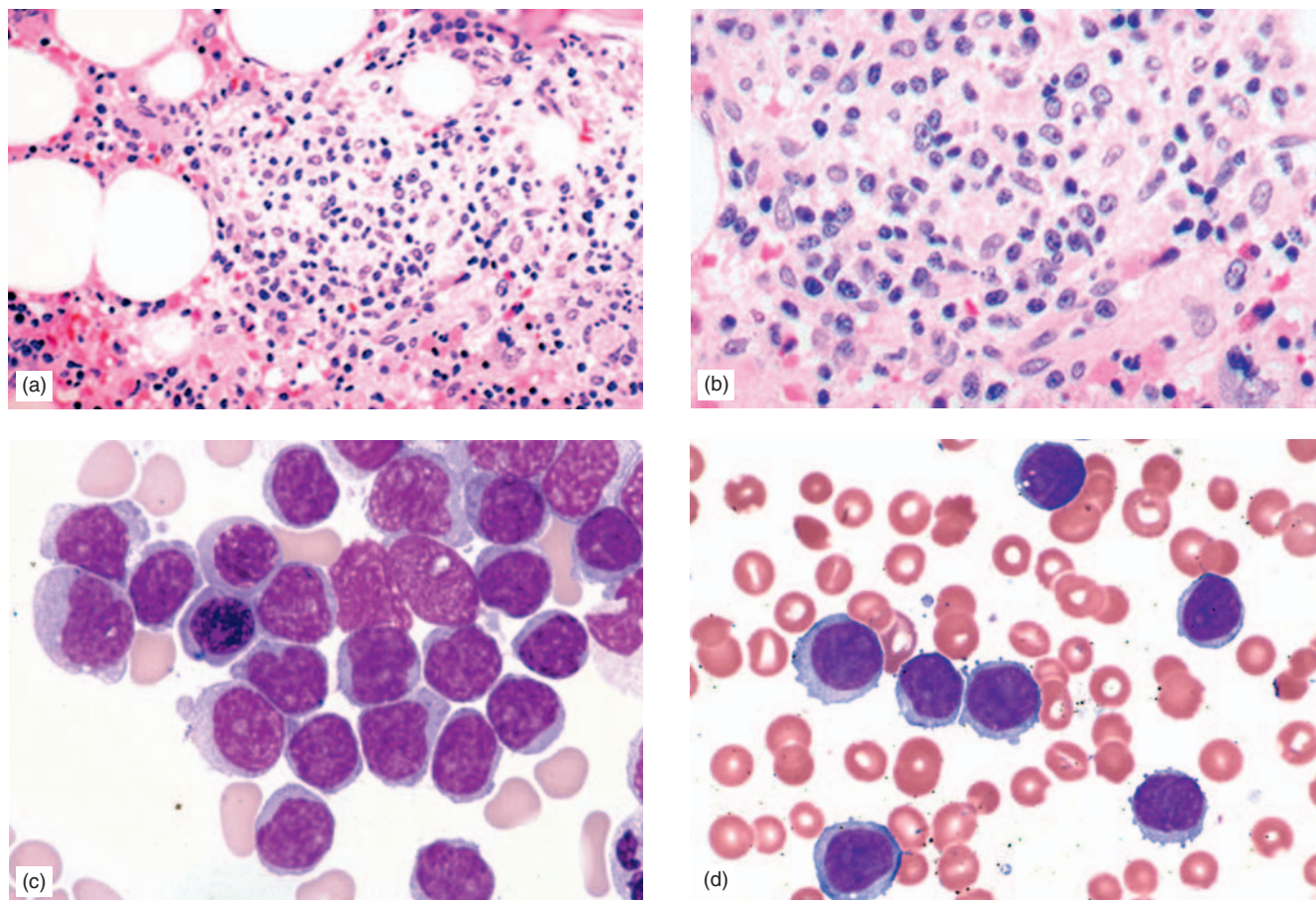


FIGURE 15.20 Polymorphocytic leukemia. (a and b) Bone marrow biopsy section, (c) bone marrow smear, and (d) blood smear. From Ref. [91] by permission.

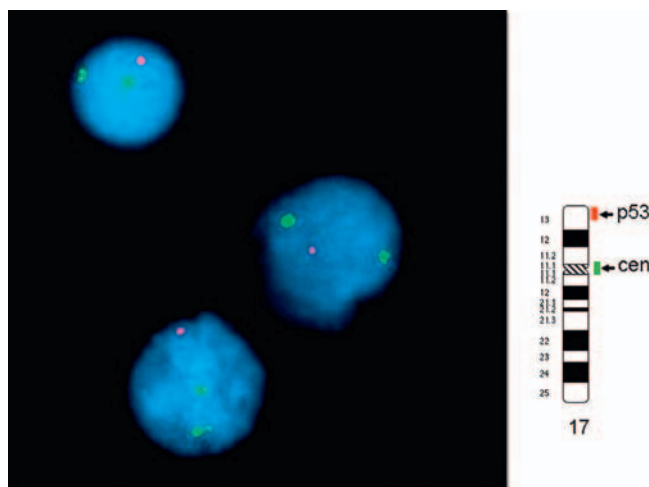


FIGURE 15.21 Deletion of *p53* (red signal) in B-cell polymorphocytic leukemia.

Other reported cytogenetic abnormalities include *del*(6q), *t*(6;12), and *t*(8;14) [68, 77]. B-PLL will usually show clonal immunoglobulin gene rearrangements just like the other B-cell malignancies.

Clinical Aspects

B-PLL is extremely rare and accounts for about 1% of all chronic lymphoid leukemias. It tends to affect elderly patients, usually >60 years old. The male:female ratio is >1. The characteristic features of B-PLL include a markedly elevated lymphocyte count, often >100,000/ μ L, massive splenomegaly, and minimal or no peripheral lymphadenopathy [68–70]. Anemia and thrombocytopenia are frequent findings. Serous effusions and central nervous system involvements are infrequent [68, 78]. B-PLL has an aggressive clinical course with a median survival time of about 3 years [79]. A complete response to alemtuzumab (anti-CD52 antibody) has been recently reported in a patient with B-PLL [80]. Transformation of B-PLL to diffuse large cell lymphoma (Richter syndrome) has been observed [81].

Differential Diagnosis

The differential diagnosis of B-PLL is with atypical CLL (CLL/PLL), HCL variant, MCL, and splenic marginal zone B-cell lymphoma.

The characteristic feature of B-PLL is >55% prolymphocytes in the peripheral blood lymphocyte count, whereas in CLL/PLL the prolymphocytes account for 10–55% of the lymphoid cells (Figure 15.22). The immunophenotype of CLL/PLL in most instances is similar to that of CLL, show coexpression of CD5 and CD23 and lack of FMC7, whereas the leukemic cells in *de novo* B-PLL lack CD5 and CD23 expression, but express FMC7.

Splenomegaly, similar to B-PLL, is one of the clinical hallmarks of HCL. But the lymphocyte count in HCL is low, normal, or modestly elevated. In a variant of HCL, leukemic cells morphologically mimic prolymphocytes. But the hairy cells are positive for tartrate-resistant acid phosphatase (TRAP) and express CD103, whereas B-PLL cells are negative for TRAP and CD103.

As briefly discussed earlier, the neoplastic cells in a group of patients with splenomegaly and markedly elevated peripheral blood lymphocyte count show morphologic features identical to prolymphocytes with cytogenetic evidence of t(11;14). These disorders were originally considered as B-PLL, but now most investigators consider these as a variant of splenic MCL.

Splenic marginal zone B-cell lymphomas similar to B-PLL usually show massive splenomegaly. But the peripheral blood lymphocyte count is usually much lower, the neoplastic cells are morphologically different from prolymphocytes (see the following section).

LYMPHOPLASMACYTIC LYMPHOMA/ WALDENSTROM MACROGLOBULINEMIA

Lymphoplasmacytic lymphoma/Waldenström macroglobulinemia (LPL/WM) is a mature B-cell lymphoproliferative disorder consisting of small lymphocytes, plasmacytoid lymphocytes, and plasma cells with the production of monoclonal IgM [1, 2, 82]. The primary sites of the involvement are bone marrow, lymph nodes, and spleen.

Etiology and Pathogenesis

The etiology and pathogenesis of the LPL/WM are not known. In several studies, the development of LPL/WM has been attributed to hepatitis C infection [83–85]. Occupational exposure to leather, rubber, dyes, and paints has been associated with LPL/WM in occasional cases [86]. Identification of family clusters and detection of the disease in identical twins suggest a genetic predisposition [87–89].

Recent studies demonstrate that in the majority of LPL/WM cases, class switch recombination (CSR) does not occur, suggesting that the neoplastic cells are either constitutively unable to carry out CSR or are prevented from doing so [90].

Pathology

Morphology and Laboratory Findings

The bone marrow biopsy sections show a nodular or diffuse infiltration of the marrow by lymphocytes, plasmacytoid

lymphocytes, and plasma cells in various proportions. Scattered prolymphocytes and immunoblasts are usually present (Figures 15.22–15.24) [91–93]. The mixed lymphoplasmacytic population is more clearly demonstrated in the bone marrow smears, which may also show increased numbers of mast cells. Plasma cells may show Ig-containing nuclear inclusions (Dutcher bodies) or cytoplasmic inclusions (Russell bodies). Circulating neoplastic cells may be seen in the peripheral blood smears, some with plasmacytoid features, but lymphocyte count is not as high as observed in CLL (Figure 15.23c). The red blood cells show rouleaux formation or evidence of agglutination (cryoglobulinemia). Moderate to severe anemia is noted in up to 80% of the patients. Monoclonal IgM serum levels are usually >3 g/dL (75% kappa light chain restricted).

The lymph node biopsy sections show a diffuse involvement with sheets of lymphocytes admixed with plasmacytoid lymphocytes and plasma cells. Dutcher and/or Russell bodies may be present. The sinuses are often open with histiocytes engulfing immunoglobulin molecules. Prolymphocytes and immunoblasts are dispersed throughout the lesion, but proliferation centers, characteristics of CLL/SLL, are often lacking. Splenic involvement is often diffuse with the infiltration of both white and red pulps [1].

Immunophenotype

In the majority of LPL/WM cases, the neoplastic cells show characteristic immunophenotypic features of post-germinal B-cells and express CD19, CD20, CD22, CD79a, and FMC7 [1, 2, 81, 94]. These cells express plasma-cell-associated markers CD38 and CD138, are negative for CD5 and CD10, but may show dim CD23 expression [94]. They are IgM-positive but lack IgD expression [1, 81].

Cytogenetic and Molecular Studies

Deletion of 6q21–q23 is the most common chromosomal aberration in LPL/WM, reported in 40–70% of the patients (Figure 15.25) [84, 95]. Some patients may demonstrate t(9;14)(p13;q32). This translocation puts *PAX5* and *IGH* genes in juxtaposition. *PAX5* encodes B-cell-specific activator protein (BSAP), which is an important regulator of B-cell proliferation and differentiation. Other reported chromosomal abnormalities include trisomies or structural aberrations of chromosomes 10, 11, 12, 15, 20, and 21 [96, 97]. The LPL/WM cells show clonal rearrangement of immunoglobulin heavy and light chains with somatic mutation of the V-region genes [98]. As noted earlier, documentation of somatic V-region hypermutation, a property particularly of more mature B-cells that have passed through the germinal center, requires subcloning and sequencing studies, which are not routinely available in most clinical molecular pathology laboratories. Mutation of the *p53* tumor suppressor gene has also been described in some cases.

Clinical Aspects

LPL/WM is a rare disorder accounting for 1–1.5% of the lymphoid malignancies. The median age is about 65 years with <1% of the patients under 40 years of age.

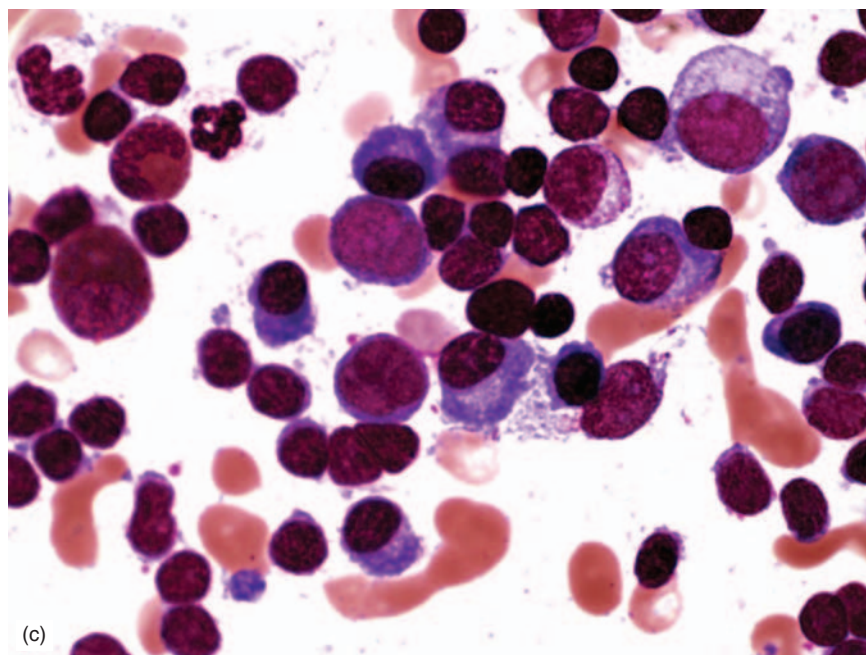
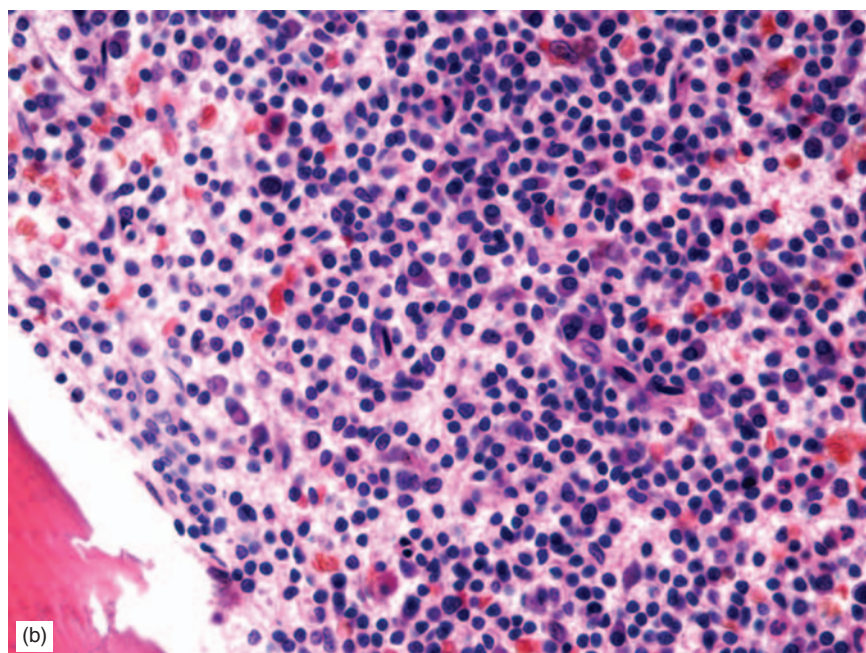
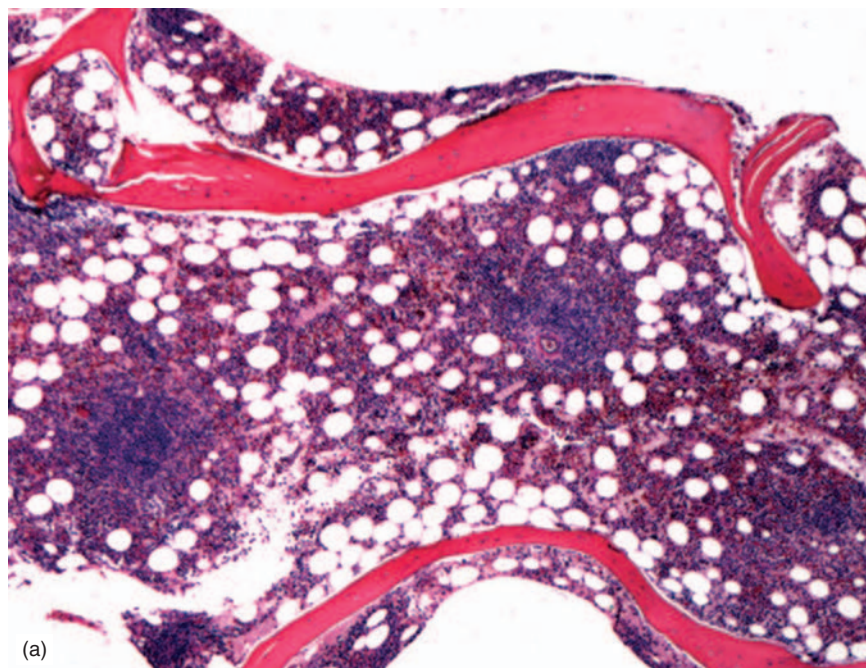


FIGURE 15.22 Lymphoplasmacytic lymphoma. The biopsy section demonstrates a nodular bone marrow involvement with a lymphoplasmacytic infiltrate: (a) low power and (b) high power views. The bone marrow smear shows a mixture of lymphocytes, plasmacytoid lymphocytes, and plasma cells (c).

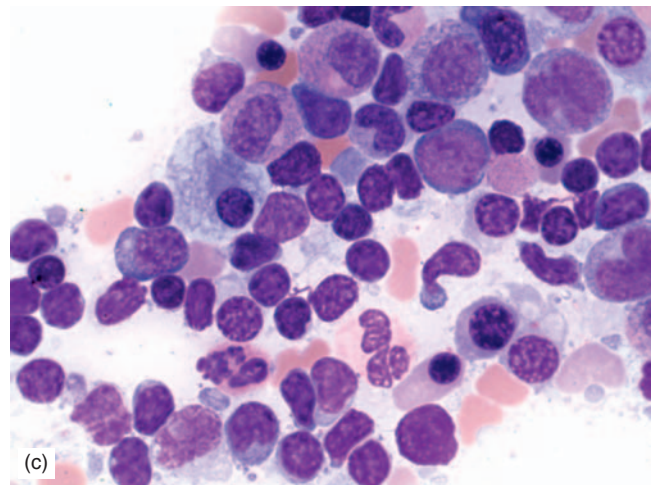
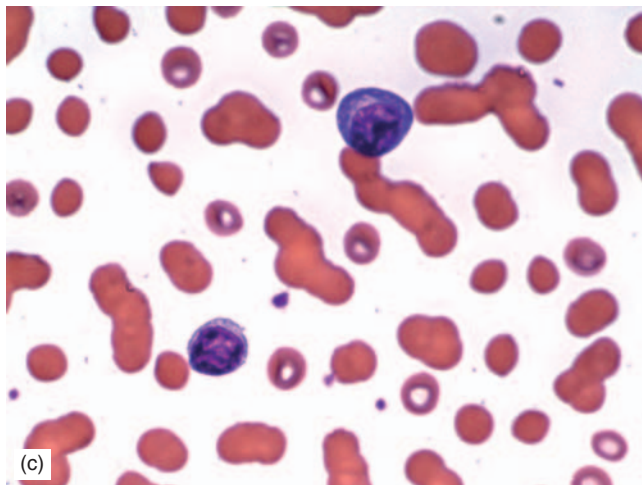
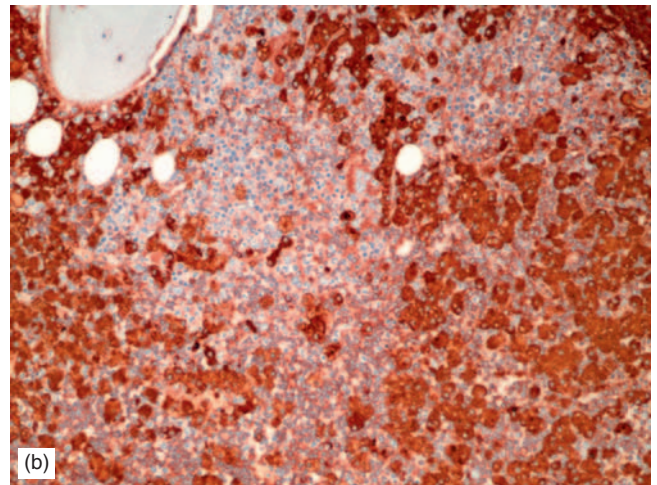
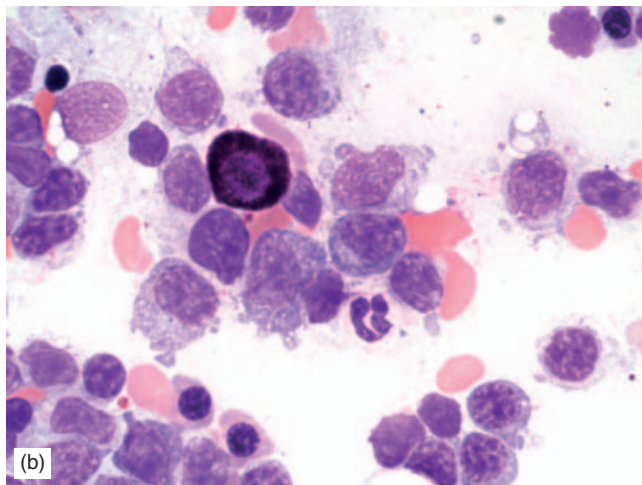
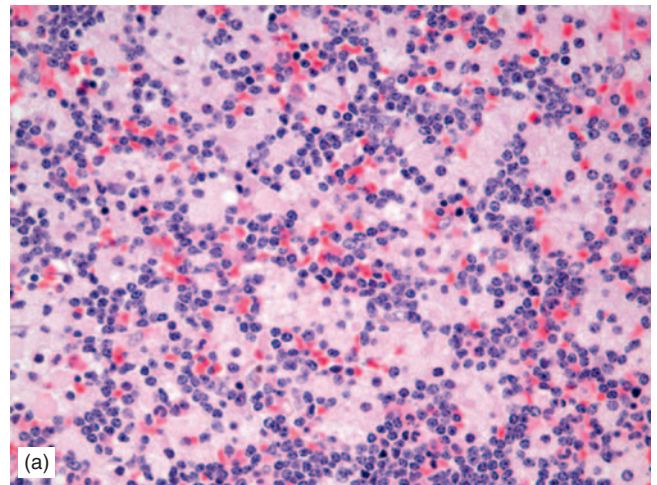
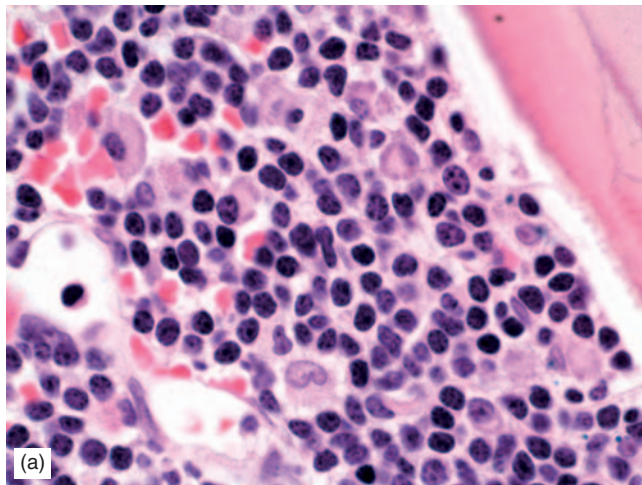
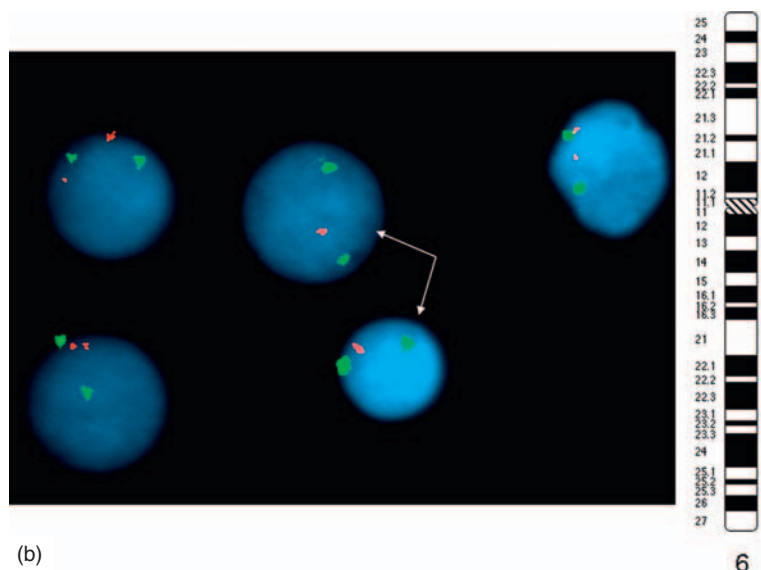


FIGURE 15.23 Lymphoplasmacytic lymphoma. The biopsy section and bone marrow smear demonstrate a lymphoplasmacytic infiltrate (a and b). The peripheral blood smear shows circulating plasma cells and red cell rouleaux formation (c).

FIGURE 15.24 Lymphoplasmacytic lymphoma. Bone marrow biopsy section demonstrates a lymphoplasmacytic infiltrate with aggregates of plasma cells with abundant pale vacuolated cytoplasm (a). These plasma cells express cytoplasmic Ig kappa light chain by immunoperoxidase stain (b). Bone marrow smear displays a lymphoplasmacytic infiltrate (c). From Ref. [91] by permission.



FIGURE 15.25 G-banded karyotype (a) with a deletion of 6q and confirmed on interphase cells by FISH (b) with a 6q-specific probe (red signal) (arrows).



Men account for approximately 60% of the patients [82, 99, 100].

Clinical symptoms include fatigue, bleeding, and hyperviscosity-related neuropathy, such as headache, vertigo, blurring or loss of vision, diplopia, or ataxia. Bone pain is rare and <5% of the patients have lytic bone lesions. Lymphadenopathy and splenomegaly are reported in 4–30% of the patients. Cryoglobulinemia, autoimmune hemolytic anemia and/or thrombocytopenia, and coagulopathies may occur [101]. Amyloidosis and erythematous urticarial skin vasculitis (Schnitzler syndrome) have been reported in some LPL/WM patients [102].

The Third International Workshop on Waldenstrom's Macroglobulinemia recommended treatment for patients with hemoglobin levels <10 g/dL, platelet count <100,000/mL, bulky adenopathy or organomegaly, symptomatic hyperviscosity, amyloidosis, and cryoglobulinemia [103]. A variety

of therapeutic regimens are available including rituximab (anti-CD20) and combination chemotherapy with or without rituximab [103–105].

Differential Diagnosis

The differential diagnosis of LPL includes small B-cell lymphomas, such as CLL/SLL and MCL, plasma cell myeloma, and monoclonal gammopathy of undetermined significance (MGUS) [105]. The immunophenotypic and cytogenetic features of LPL/WM, CLL, and MCL are presented in Table 15.5. Unlike CLL cells, the neoplastic cells of LPL are negative for CD5 and CD23 and may express CD138. The major distinguishing features of MCL are CD5+, BCL-1+, CD23–, and t(11;14). LPL cells are CD5 negative and show no t(11;14), but frequently demonstrate del(6q21–23). Plasma cell

TABLE 15.5 Morphologic, immunophenotypic, and cytogenetic characteristics of lymphoplasmacytic lymphoma (LPL), splenic marginal zone lymphoma (SMZL), and hairy cell leukemia (HCL).

Features	LPL	SMZL	HCL
Spleen			
Primary involved area	White pulp	White pulp	Red pulp
Pattern	Nodular	Nodular	Diffuse
Bone marrow	Nodular, diffuse or interstitial	Mostly intrasinusoidal	Mostly interstitial
Cytology	Small lymphocytes, plasmacytoid lymphocytes	Medium-sized lymphocytes, polar villous projections	Medium-sized lymphocytes, hairy projections
Immunophenotype	IgM+, CD19+, CD20+, CD22+, CD79+, FMC7+, CD23±, CD5−, CD10−, CD25−, CD103−, TRAP−	IgM+, IgD+, CD19+, CD20+, CD22+, CD79a+, CD5−, CD10−, CD23−, CD25−, CD103−, TRAP−	IgM+, CD19+, CD20+, CD22+, CD79a+, FMC7+, CD11c+, CD25+, CD103+, TRAP+, CD5−, CD10−, CD23−
Cytogenetics	del(6)	del(7)(q21→q32)	None

disorders are comprised predominantly of plasma cells, rarely involve lymph nodes or spleen, and show a non-IgM monoclonal serum paraprotein (see Chapter 16).

SPLENIC MARGINAL ZONE LYMPHOMA

Splenic marginal zone lymphoma (SMZL) or splenic lymphoma with villous lymphocytes is a B-cell neoplasm of small to medium-sized lymphocytes presumably arising from the marginal zone of the splenic white pulp. Characteristic features include splenomegaly, bone marrow infiltration with intrasinusoidal pattern, moderate peripheral blood lymphocytosis with the presence of villous lymphocytes, and a relative indolent course [1, 2, 106–110].

There are two other types of marginal zone-related B-cell non-Hodgkin lymphomas: nodal marginal zone B-cell lymphoma and extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) type. These two distinct clinicopathologic entities are discussed separately in the following sections.

Etiology and Pathogenesis

The etiology and pathogenesis of SMZL are not known. The possibility of environmental factors initiating *in vivo* somatic mutation of *IGVH* gene has been entertained [111]. The increased incidence of SMZL in patients infected with hepatitis C virus suggests a role for a viral antigen epitope in the B-cell selection and the development of SMZL [1, 112].

Allelic loss of chromosome 7q (deletions of 7q22–32) is frequently observed in SMZL [113]. These alterations lead to dysregulation of cyclin-dependent kinase 6 (*CDK6*) gene, possibly playing a role in the pathogenesis of SMZL. Also, the 7q deletion may play a role in the inactivation of *p53* tumor suppressor gene.

Pathology

Morphology

The spleen is enlarged with a median weight of 1,750g. The cut surface usually shows multiple gray-tan nodules of various sizes. Diffuse involvement is rare [1, 2, 110]. In the early stages of involvement, the splenic sections show enlarged white pulps with the expansion of marginal zones merging into one another. In the center of the nodules, usually there is a remnant of germinal center surrounded by mantle cells and expanded marginal zones (Figure 15.26). Mantle cells are small with scanty cytoplasm and slightly irregular nuclei. The cells surrounding mantle cells in the marginal zone are larger with more dispersed chromatin and abundant pale cytoplasm. Admixed with these cells are scattered centroblasts and immunoblasts. There is infiltration of neoplastic cells into the red pulp (Figure 15.27). In later stages, eventually, the white pulp expansion and red pulp infiltration create sheets of neoplastic cells, making the separation of white and red pulps unclear [114].

The hilar splenic lymph nodes are commonly involved. The peripheral lymph nodes are affected less frequently. There is partial effacement of nodal architecture with infiltrating neoplastic nodules. Some of the nodules may contain a central reactive follicle. Sinuses are usually spared. Some cases may show complete effacement of the nodal architecture.

Bone marrow is commonly involved. The pattern of involvement is intrasinusoidal, interstitial, nodular, paratrabeular, or a combination of these (Figures 15.28 and 15.29). The intrasinusoidal pattern is highly characteristic of SMZL. The bone marrow smears show the presence of atypical small to medium-sized lymphocytes with abundant cytoplasm, round or irregular nuclei, and condensed chromatin. Some of the lymphocytes may show villous cytoplasmic projections, which are often polar (Figure 15.29c).

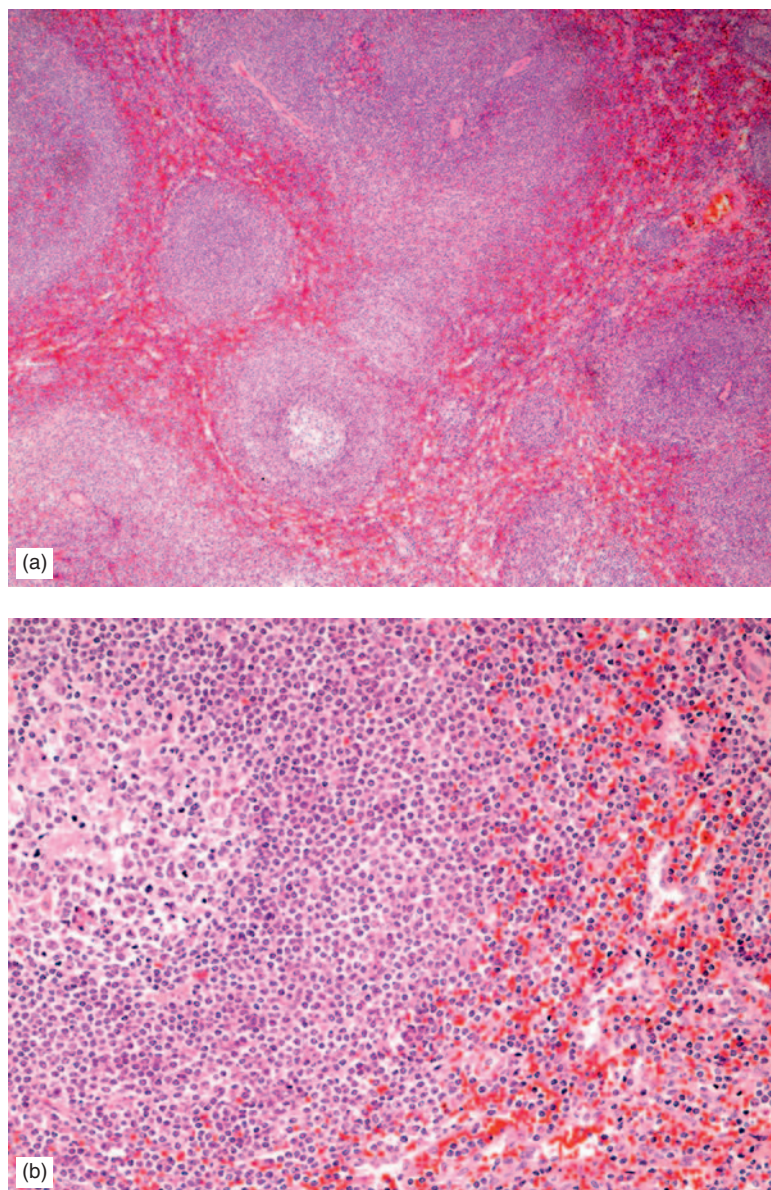


FIGURE 15.26 Splenic marginal zone B-cell lymphoma demonstrating the expansion of marginal zone of the white pulp in the spleen: (a) low power and (b) high power views.

There is usually a moderate peripheral blood lymphocytosis with various proportions of atypical lymphoid cells. These cells are morphologically similar to those described in the bone marrow smears and may or may not show polar villous projections. Those without villous projections may appear plasmacytoid. Scattered larger cells with prominent nucleoli may be present.

The liver is involved in the majority of the cases. The portal tracts are the predominant sites of infiltration. Skin, pleura, and soft tissue involvements have been rarely reported.

Immunophenotype

The neoplastic cells express surface IgM and IgD and show positivity for B-cell-associated markers, such as CD19, CD20, CD79a, FMC7, and *PAX5* [1, 2, 110]. They are negative for CD5, CD10, CD23, CD25, CD103, BCL-1, and BCL-6.

They express BCL-2 and usually lack the expression of CD43 and TRAP. Results for DBA44 staining are variable.

Cytogenetic and Molecular Studies

As mentioned earlier, allelic loss of chromosome 7q (deletions of 7q22-32) and dysregulation of the *CDK6* gene have been reported in some cases of SMZL [113]. Abnormalities of chromosome 14q32, harboring the *IGH* gene, have also been reported in SMZL in the forms of t(6;14) and t(9;14) (Figure 15.30). The earlier reports of SMZL with t(11;14) and *BCL-1* gene rearrangement are now believed to represent MCL. Trisomy 3 is observed in some cases of SMZL, but it is more frequent in the nodal and extranodal types (Figure 15.31). Abnormalities of *p53* gene are reported in about 17% of the patients. Most SMZL cases have been associated with multiple somatic immunoglobulin variable region hypermutations, but a subset has been

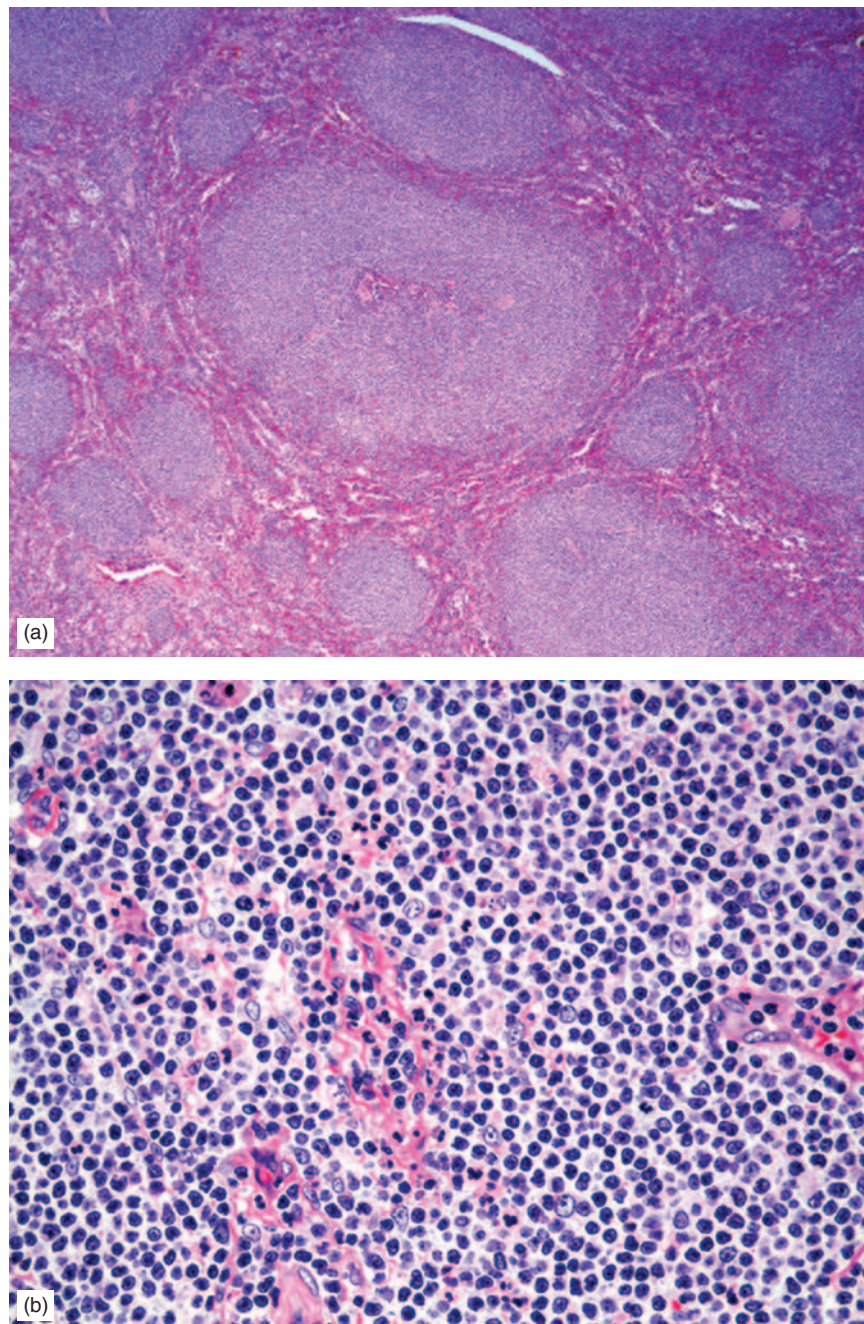


FIGURE 15.27 Splenic marginal zone B-cell lymphoma demonstrating the expansion of marginal zone into the splenic red pulp (a). The high power view shows monocytoid lymphocytes with variable amounts of cytoplasm (b).

identified without mutations that may have a different clinical course [113, 115].

Clinical Aspects

SMZL accounts for about 1–2% of non-Hodgkin lymphomas and constitutes 8–14% of lymphomas in the surgically removed spleens [106–110]. The median age is 65 years with a male:female ratio of about 1 or 1:2. Moderate to massive splenomegaly is a common feature. Hepatomegaly is observed in some patients, but peripheral lymphadenopathy

is rare. Patients usually develop mild to moderate degrees of anemia, thrombocytopenia, and neutropenia which could be attributed to bone marrow infiltration and splenic sequestration. The majority of the patients show absolute lymphocytosis. Serum immunoglobulin studies reveal a small IgM or IgG spike, usually <3g/dL in about 50% of the cases. Transformation to large cell lymphoma occasionally occurs.

Autoimmune conditions such as autoimmune hemolytic anemia, immune thrombocytopenia, lupus anticoagulants, rheumatoid arthritis, and biliary cirrhosis have been observed in association with SMZL [106–110].

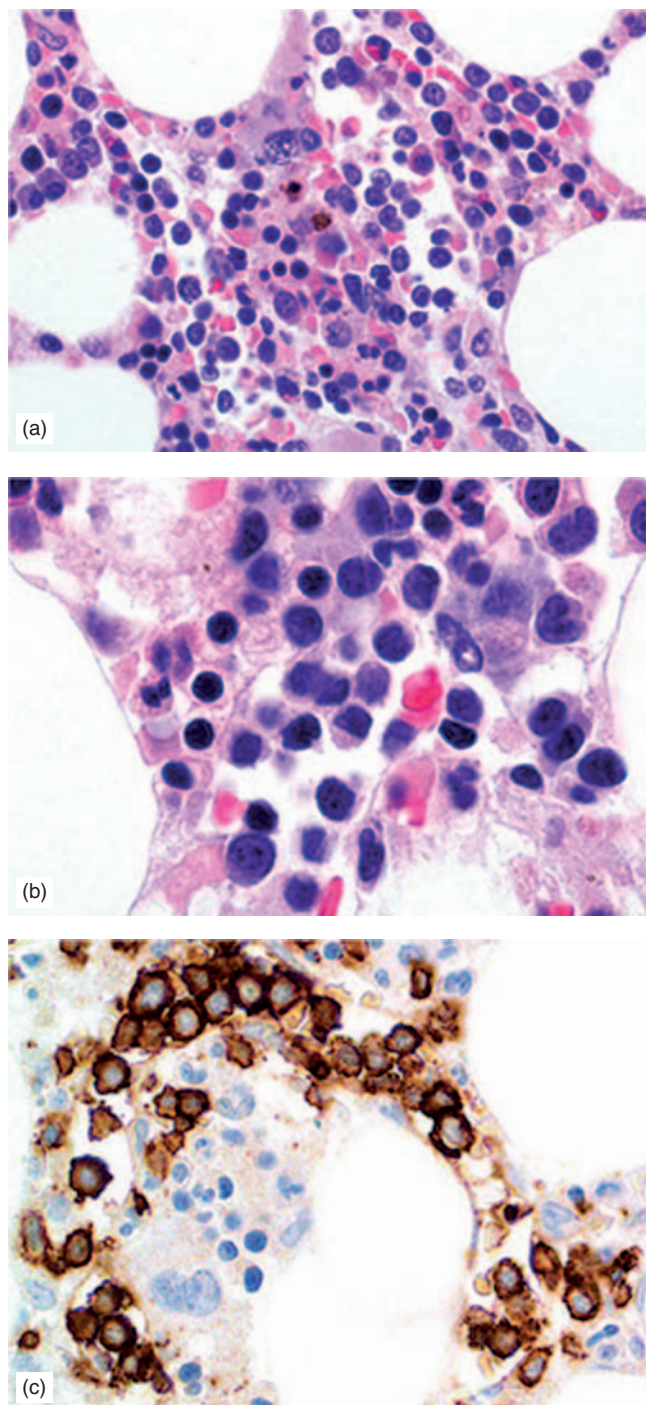


FIGURE 15.28 Bone marrow involvement in splenic marginal zone B-cell lymphoma is often sinusoidal: (a) low power and (b) high power. Immunohistochemical stains show clusters of CD20⁺ cells within the sinusoids (c). From Ref. [91] by permission.

The SMZL is considered a low-risk lymphoma with an indolent clinical course. The overall survival is >70% at 10 years with complete remission rate of 80%. The treatment strategies include (1) no therapy, (2) splenic irradiation, (3) splenectomy, (4) chemotherapy, and (5) treatment with anti-CD20 [110, 116–118].

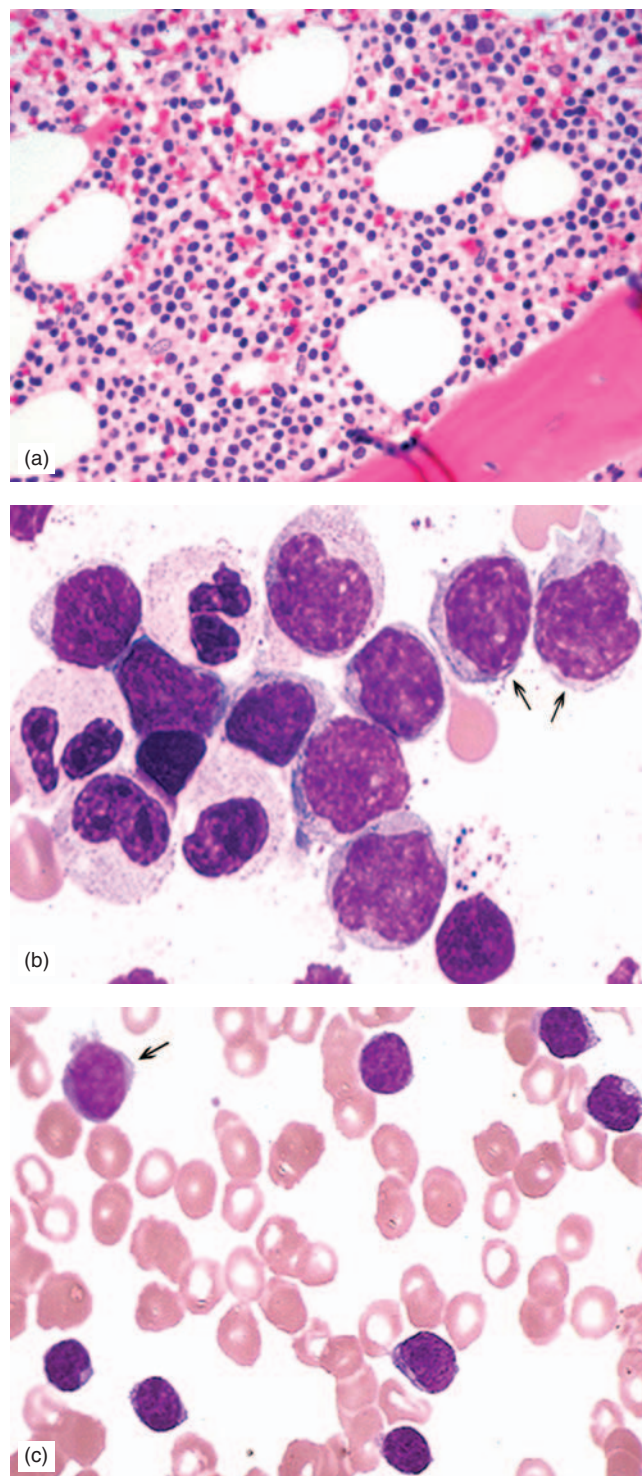


FIGURE 15.29 Bone marrow involvement in splenic marginal zone B-cell lymphoma: (a) biopsy section, (b) bone marrow smear, and (c) blood smear. Some lymphocytes show polar cytoplasmic projections.

Differential Diagnosis

The differential diagnosis of SMZL includes HCL, LPL, MCL, FL, and CLL/SLL. A summary of morphologic, immunophenotypic, and cytogenetic characteristics of SMZL, HCL, and LPL is presented in Table 15.5.

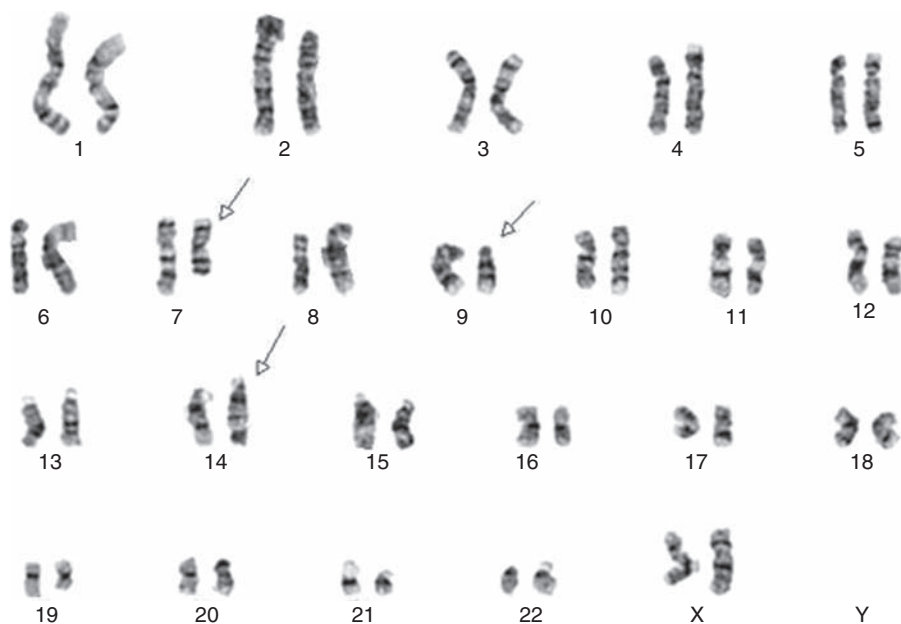


FIGURE 15.30 G-banded karyotype of the tumor cells in a patient with splenic marginal zone B-cell lymphoma demonstrating 46,XX,del(7)(q32), t(9;14)(p13;q32).

SMZL involves the splenic white pulp and often displays a nodular pattern, whereas HCL diffusely involves the red pulp with atrophy of the white pulp. Bone marrow fibrosis and interstitial infiltration are common features in HCL, whereas in SMZL marrow fibrosis is infrequent and intrasinusoidal infiltration is the characteristic feature. Unlike SMZL cells, HCL cells are TRAP-positive and express CD25 and CD103.

Overall SMZL cells are larger and have more abundant cytoplasm than the neoplastic cells in LPL. The IgM serum levels are usually <3 g/dL in SMZL and >3 g/dL in LPL. SMZL cells express IgM and IgD, whereas LPL cells are only IgM-positive. The primary cytogenetic abnormalities in SMZL are deletions of 7q22-32 and trisomy 3, whereas del(q21-q23) and t(9;14) are the cytogenetic characteristics of LPL.

The neoplastic cells in MCL are usually smaller and unlike SMZL cells, express CD5 and BCL-1. MCL has a much higher frequency of peripheral lymphadenopathy than SMZL. The cytogenetic hallmark of MCL is t(11;14).

The neoplastic cells in FL consist of a mixture of smaller centrocytes and larger centroblasts. These cells, unlike SMZL cells, express CD10 and BCL-6. The cytogenetic hallmark of FL is t(14;18).

CLL/SLL primarily consists of small mature lymphocytes with scanty cytoplasm. These cells, unlike SMZL cells, coexpress CD5 and CD23, are usually negative for FMC7, show dim expression of CD20, CD22, and CD79a, and have different cytogenetic profiles (see Table 15.5).

EXTRANODAL MARGINAL ZONE B-CELL LYMPHOMA OF MUCOSA-ASSOCIATED LYMPHOID TISSUE

Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) represents

a neoplastic B-cell infiltrate which extends to the adjacent epithelium [1, 2, 119–122]. The cells are polymorphic, consisting of small lymphocytes, marginal zone cells, monocytoid cells, and often plasma cells. Scattered immunoblasts and centroblast-like cells are also present.

Etiology and Pathogenesis

A strong association has been found between MALT lymphoma and certain autoimmune disorders and infections, such as Sjogren syndrome, Hashimoto thyroiditis, hepatitis C virus infection, *Helicobacter gastritis*, and *Borrelia afzelii* infection of skin [123–126]. According to Isaacson, the reactive MALT lymphoma in autoimmune conditions or infections in stomach, salivary gland, thyroid gland, lung, and other tissues provide the substrate for the development of lymphoma. In most instances, treatment of *H. pylori* in gastric MALT lymphoma results in regression of early lesions [123]. Similarly, a proportion of cutaneous MALT-type lymphomas may regress by antibiotic therapy for *Borrelia* infection [125, 126].

The juxtaposition of *BIRC3* (formerly *API2*)(11q21) and *MALT1*(18q21) genes associated with t(11;18), the most common structural abnormality observed in MALT lymphoma (discussed later), may play a role in the pathogenesis of this disorder [122, 127]. The *API2-MALT* translocation increases NF-kappaB activation. NF-kappaB is a transcription factor that plays a role in preventing TNF- α -induced cell death [128, 129].

Pathology

Morphology

The most common site of MALT lymphoma is the gastrointestinal tract accounting for about 50% of the cases. Other sites of involvement in order of frequency include lung, salivary

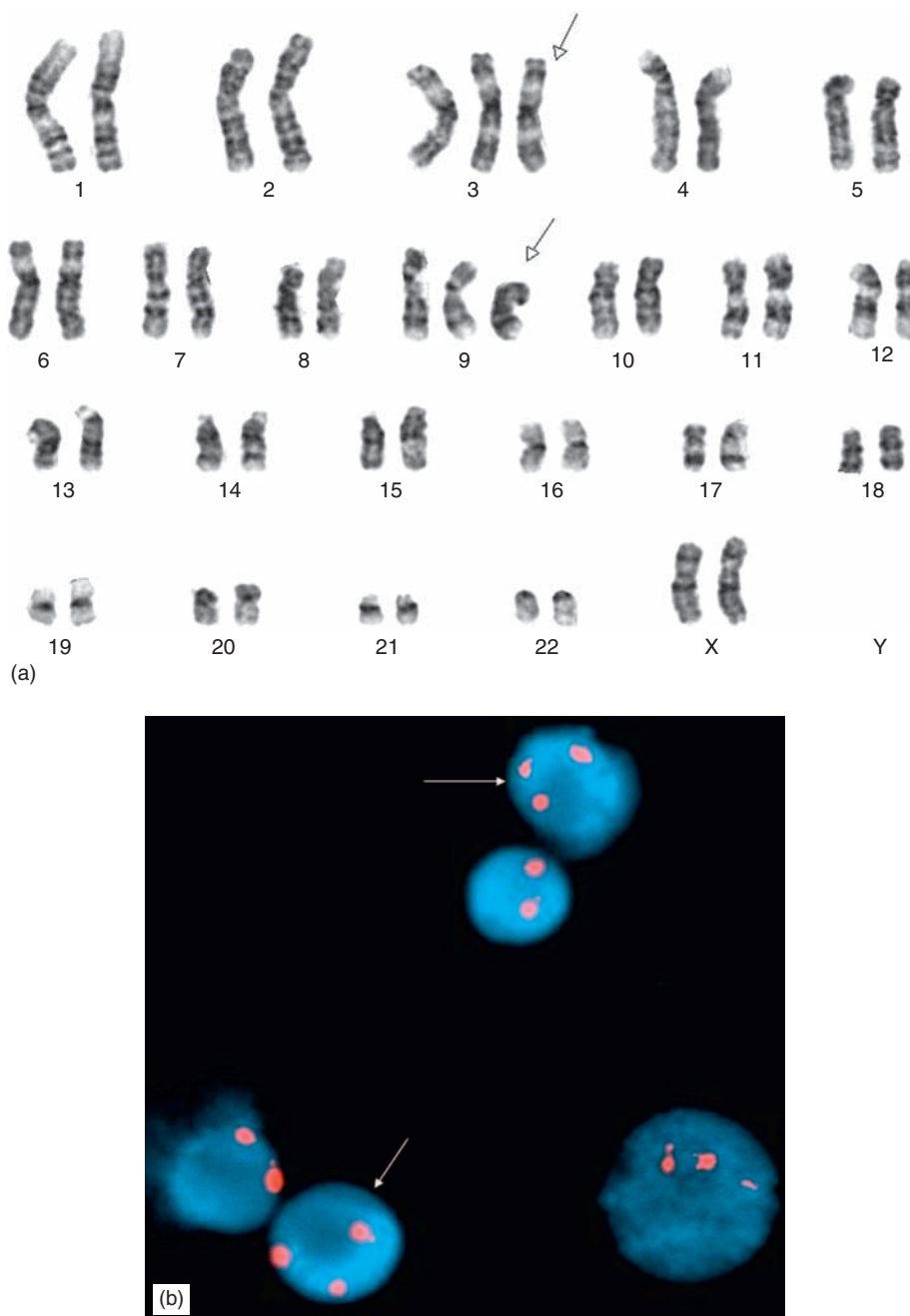


FIGURE 15.31 (a) G-banded karyotype of the tumor cells in a patient with splenic marginal zone B-cell lymphoma demonstrating 48,XX, +3, +9. Trisomy 3 is also demonstrated by FISH analysis (b).

glands, ocular adenexae, skin, thyroid, and breast [1, 2, 122, 130–132]. The involved tissues show a lymphomatous infiltrate around reactive follicles spreading into the surrounding areas. The MALT lymphoma cells are polymorphous consisting of various proportions of small lymphocytes, marginal zone (centrocyte-like) cells, monocytoid B-cells, and plasma cells. Scattered blast cells (centroblast- or immunoblast-like) are often noted, but if they are in sheets or large clusters, a diagnosis of large cell lymphoma should be made. Occasional follicles may show “colonization” by marginal zone or monocytoid B-cells. The epithelial tissue is characteristically infiltrated by the neoplastic cells, forming *lymphoepithelial lesions*

(Figure 15.32). Lymphoepithelial lesion is defined as infiltrative aggregates of ≥ 3 marginal zone cells in the epithelium with distortion or destruction of the epithelial structure [1].

Lymph node or bone marrow involvement is reported in up to 25% of the cases in some studies. Peripheral blood is usually not involved in initial stages.

Immunophenotype

The immunophenotypic features of MALT lymphoma are similar to SMZL, except that MALT lymphoma cells express surface IgM (less often IgG or IgA) but lack IgD,

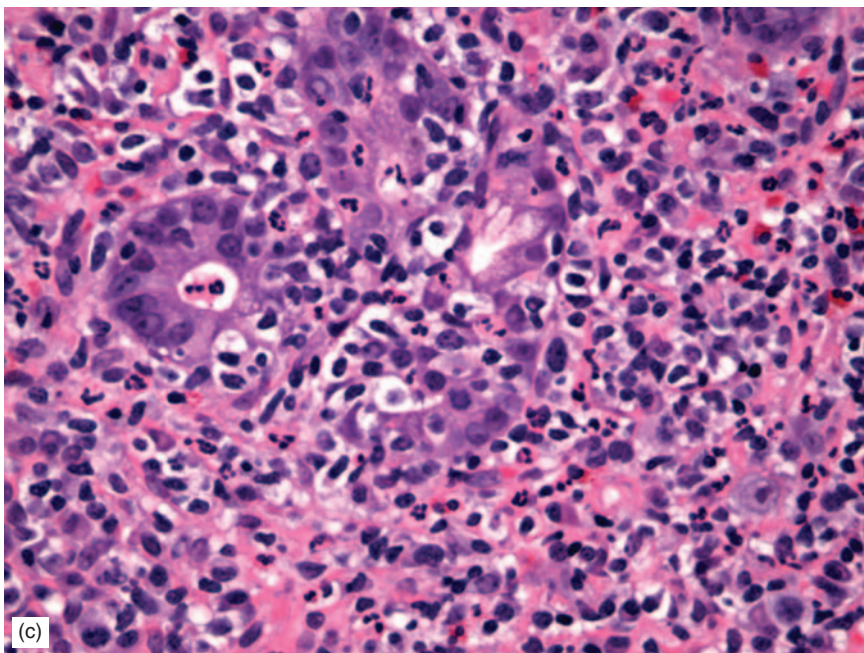
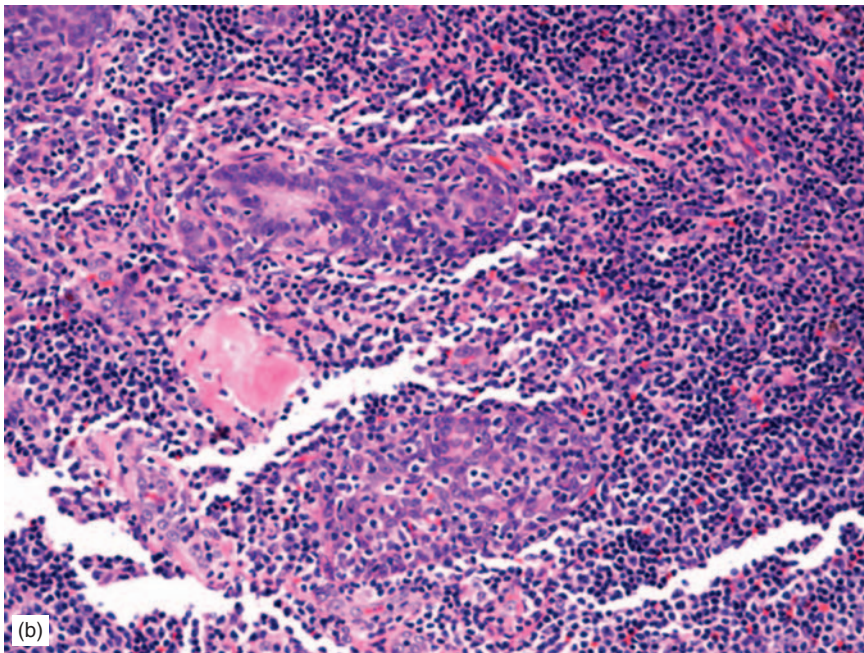
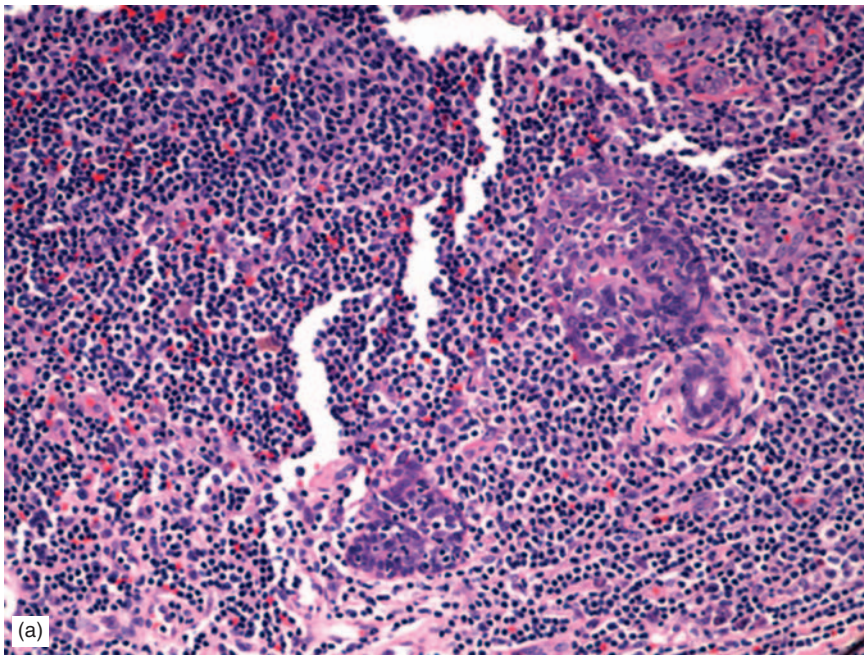


FIGURE 15.32 Lymphoepithelial lesions in the case of gastric MALT-type lymphoma: (a) low power, (b) intermediate power, and (c) high power views.

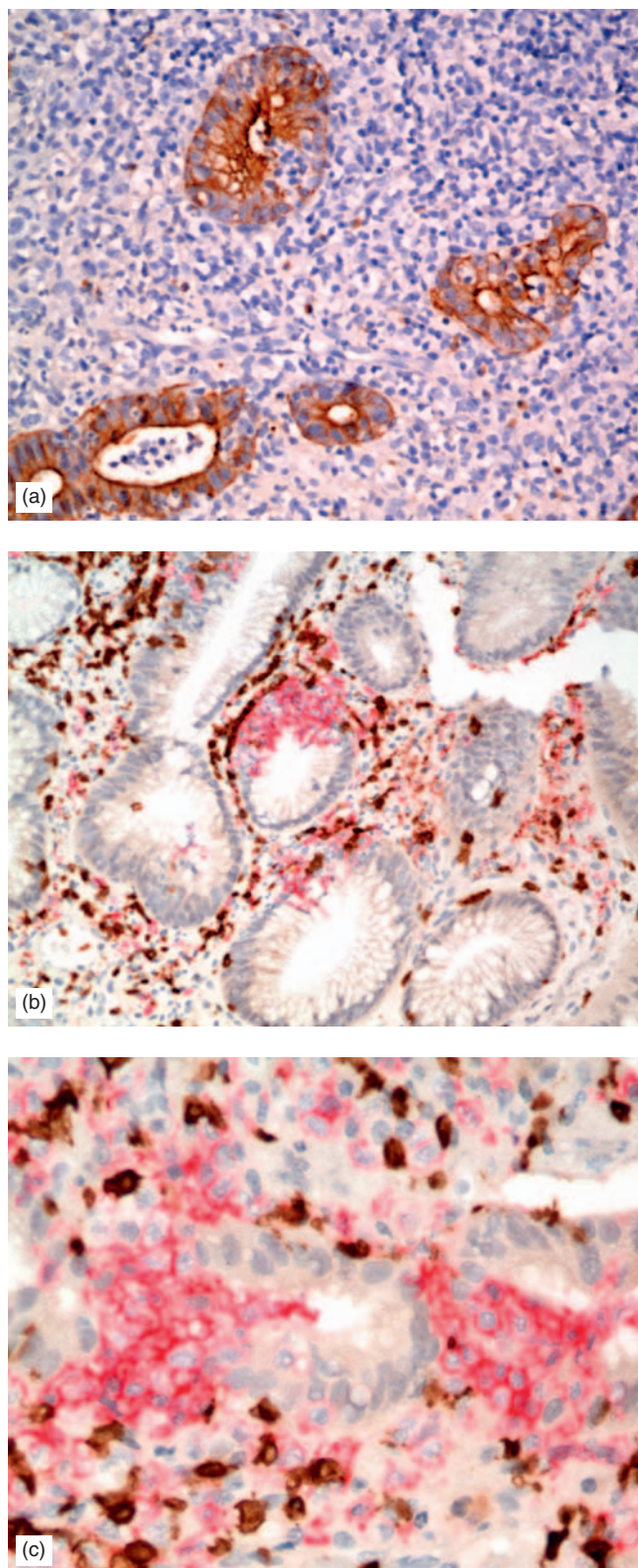


FIGURE 15.33 Immunohistochemical stains in MALT-type lymphoma. (a) A cytokeratin stain highlights glandular structures infiltrated by lymphocytes. (b) The infiltrating lymphocytes are predominantly CD20+ (red stain): (b) intermediate power and (c) high power views.

and about 40–60% of the cases show cytoplasmic Ig pointing to plasmacytic differentiation. The MALT lymphoma cells are positive for CD19, CD20, and CD79a, and negative for CD5, CD10, CD23, and BCL-1 (Figure 15.33) [1, 2, 121, 122].

Cytogenetic and Molecular Studies

The most common cytogenetic structural abnormalities reported in MALT lymphoma are $t(11;18)(q21;q21)$ and trisomy 3 found in about 25–35% of the cases (Figure 15.34) [133, 134]. A less frequent nonrandom translocation is $t(1;14)(p22;q32)$, which has been reported in association with gastric and lung MALT lymphomas [129]. These two translocations have not been observed in SMZL or nodal marginal zone B-cell lymphoma. Histological transformation of MALT lymphoma to large cell lymphoma has been associated with $t(6;14)(p21;q32)$ pointing to the alteration of cyclin D3 expression (*CCND3*) in this process [135].

Important negative findings are lack of involvement of the *BCL-1* and *BCL-2* genes and absence of $t(11;14)(q13;q32)$ and $t(14;18)(q32;q21)$. Occasional cases may show *BCL-6* rearrangements involving chromosome 3q27 [136]. Naturally, these lesions should also show clonal immunoglobulin gene rearrangements, though this finding is often of less diagnostic significance than the other markers listed here.

Trisomy 3 is the most common numerical cytogenetic abnormality in MALT lymphoma reported in up to 60% of the cases.

Clinical Aspects

MALT lymphoma accounts for about 5% of all non-Hodgkin lymphomas. The most frequent site of involvement is stomach (50%) followed by lung (14%), salivary glands (14%), ocular adnexae (12%), skin (11%), thyroid glands (4%), and breast (4%) [1, 2, 130–132].

There is no age preference, but there is a slight female predominance. Symptoms are related to the site of involvement, such as abdominal pain (peptic ulcer disease) in the case of gastric lymphoma or Sjogren's syndrome in the case of salivary gland involvement. Systemic “B” symptoms are infrequent. Involvement of multiple mucosal sites at the time of initial diagnostic workup has been reported in up to one-third of the patients.

MALT lymphomas have a high rate of complete remission with 80% survival rate at ≥ 10 years. The therapeutic approaches include a variety of combinations of antibiotics, surgery, radiation, and chemotherapy, though universally accepted optimal therapeutic regimens based on the results of controlled studies have not yet been established [137].

Differential Diagnosis

The differential diagnosis of MALT lymphoma includes a variety of reactive lymphoproliferative disorders and small

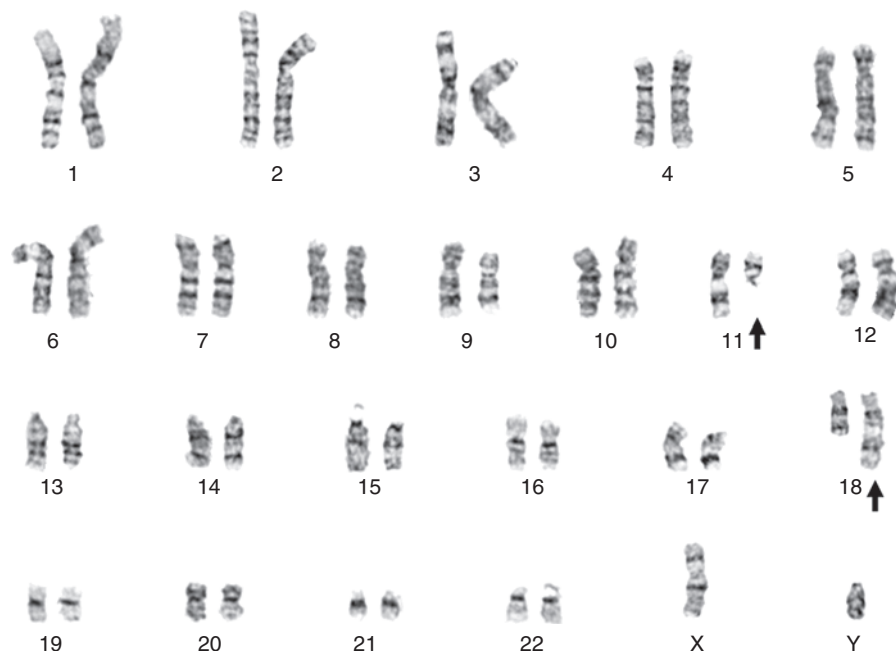


FIGURE 15.34 G-banded karyotype of tumor cells from a patient with MALT lymphoma demonstrating 46,XY,t(11;18)(q21;q21).

B-cell lymphomas. MALT lymphoma is distinguished from *H. pylori* gastritis, lymphoepithelial sialadenitis, and Hashimoto thyroiditis by the presence of destructive lymphoid infiltration of the epithelium and evidence of a monotypic B-cell population by immunophenotypic studies or molecular genetic analyses. Features distinguishing MALT lymphoma from other small B-cell lymphomas are similar to those previously discussed in SMZL.

NODAL MARGINAL ZONE B-CELL LYMPHOMA

Nodal marginal zone B-cell lymphoma (nodal MZL) is a rare, primary node-based lymphoma of marginal zone type with no evidence of extranodal disease. The diagnosis of nodal MZL should not be made in patients with Sjogren's syndrome, MALT lymphoma, or when another type of low-risk lymphoma is present in the same lymph node [1, 2, 138].

Etiology and Pathogenesis

The etiology and pathogenesis of nodal MZL are not known.

Pathology

Morphology

The neoplastic cells primarily consist of marginal zone and monocytoid B-cells infiltrating interfollicular areas of the involved lymph node. Various proportions of plasma cells and scattered blasts (centroblasts and immunoblasts) are

usually present. Follicular colonization may be present. Two morphologic subtypes have been distinguished [2]:

1. Lymph nodes with aggregates of monocytoid B-cells with a parafollicular, perivascular, and perisinusoidal distribution. Germinal centers and mantle zones are preserved. The majority of these patients show evidence of MALT lymphoma, and therefore the lymph node involvement should be considered a secondary process.
2. Lymph nodes with marginal zone B-cells infiltrating and expanding around follicles with germinal centers and shrinkage or disappearance of the mantle zones.

Bone marrow is involved in 30% of the cases. Peripheral blood involvement is rare.

Immunophenotype

Most cases show immunophenotypic features similar to MALT lymphoma. Some cases, similar to SMZL, may show IgD expression.

Cytogenetic and Molecular Studies

Trisomy 3 is observed in some cases of nodal and extranodal marginal zone B-cell lymphomas (see Figure 15.31). Immunoglobulin gene rearrangements will be present but are nonspecific.

Clinical Aspects

Nodal MZL is rare, accounting for <2% of all non-Hodgkin lymphomas [139, 140]. The median age is around 50 years. Patients show localized or generalized lymphadenopathy with no systemic "B" symptoms. Median survival exceeds 12 years.

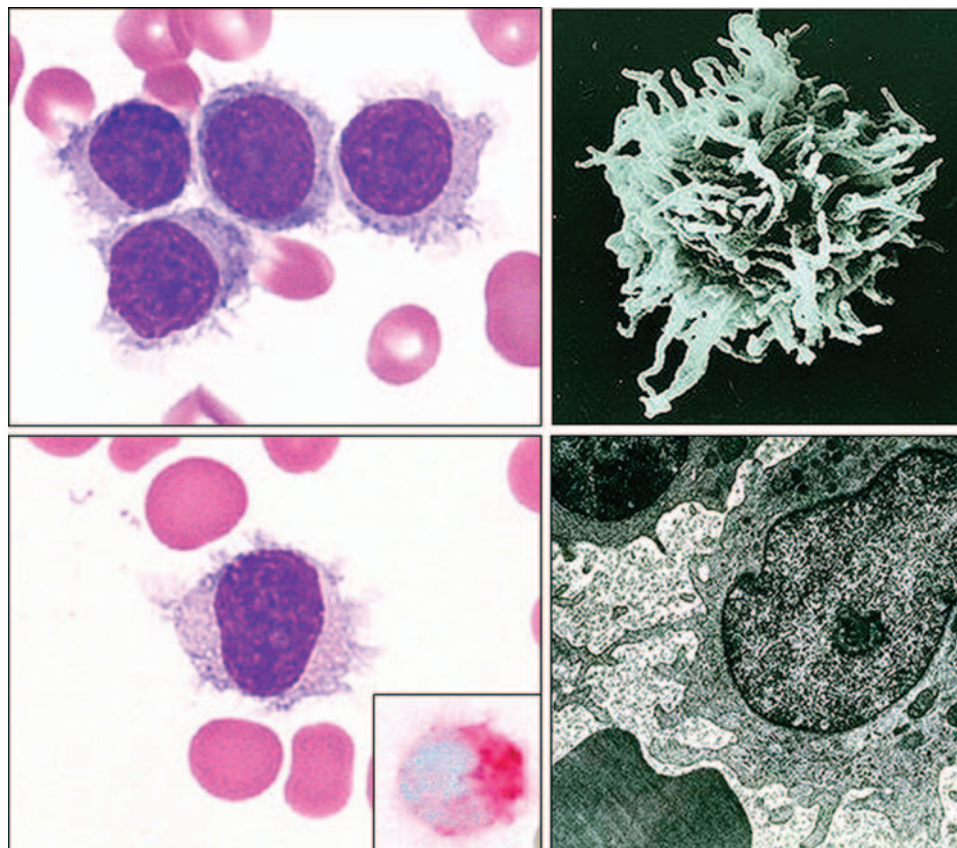


FIGURE 15.35 Hairy cell leukemia. Peripheral blood smear demonstrates neoplastic cells with cytoplasmic hairy projections (left). The inset shows a hair cell positive for tartrate-resistant acid phosphatase. Features of scanning (right top) and transmission (right bottom) electron microscopy are demonstrated. From Naeim F. (1997). *Atlas of Bone Marrow and Blood Pathology*, W.B. Saunders, Philadelphia; and Naeim F. (1980). Cytoskeletal of redistribution of surface membrane receptors in hairy cell leukemia. *Am J Clin Pathol* **74**, 660–3, by permission. From Ref. [91] by permission.

HAIRY CELL LEUKEMIA

Hairy cell leukemia, or leukemic reticuloendotheliosis, is an indolent mature B-cell lymphoid leukemia characterized by the proliferation of medium-sized lymphocytes with cytoplasmic “hairy” projections involving blood, bone marrow, spleen, and occasionally other tissues [1, 141, 142].

Etiology and Pathogenesis

The etiology and pathogenesis of HCL are not known. Environmental factors such as ionizing radiation and organic chemicals have been suggested as possible causes [143–145]. A report of siblings affected with HCL and sharing the same HLA haplotype has raised the possibility of genetic predisposition in the development of this disease in some familial cases [146]. Immunoglobulin gene rearrangement studies suggest an extrafollicular origin for the HCL cells, probably a marginal zone B-cell origin [147–149].

Pathology

Morphology

Hairy cells are larger than mature lymphocytes and show abundant pale blue cytoplasm, often with ill-defined

border (Figure 15.35) [1, 141, 142]. Cells with characteristic elongated (hairy) cytoplasmic projections are frequently identified. The nuclei are round, oval, folded, indented, or dumbbell-shaped. The nuclear chromatin is condensed but finer than in CLL cells. Rare cells may show prominent nucleoli. The hairy cytoplasmic projections are easily detected by phase contrast microscopy or by scanning and transmission electron microscopy.

Peripheral blood examination in the majority of the patients reveals pancytopenia with the presence of various proportions of hairy cells. Only about 10% of the patients show leukocytosis, and in such cases, the hairy cells account for the majority of the leukocytes. Occasionally, leukocytosis exceeds 100,000/ μ L. Monocytopenia is one of the characteristic features of HCL.

The bone marrow involvement is usually interstitial or diffuse with patchy, densely cellular areas composed of neoplastic cells (Figure 15.36). Nodular involvement is rare. In most cases, the bone marrow is hypercellular with a cellularity of >50–90%. But occasionally, the bone marrow may appear markedly hypocellular (<25%) simulating aplastic anemia (Figure 15.37). The HCL cells are relatively uniform with abundant clear cytoplasm and round, oval, or irregular nuclei, often without prominent nucleoli or presence of mitotic figures. The presence of abundant cytoplasm creates a nuclear spacing or “fried egg” pattern. Extravasated red blood cells are frequently seen.

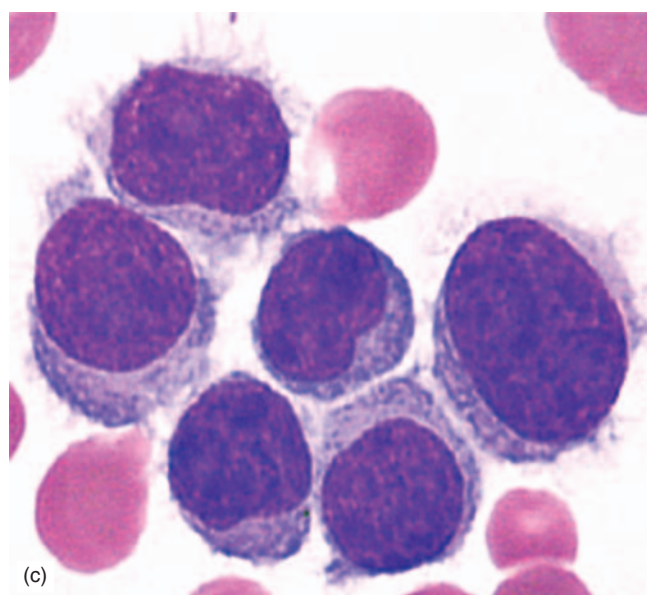
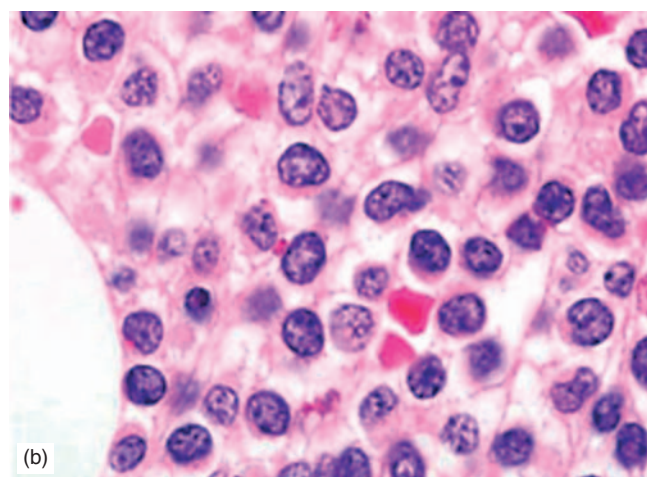
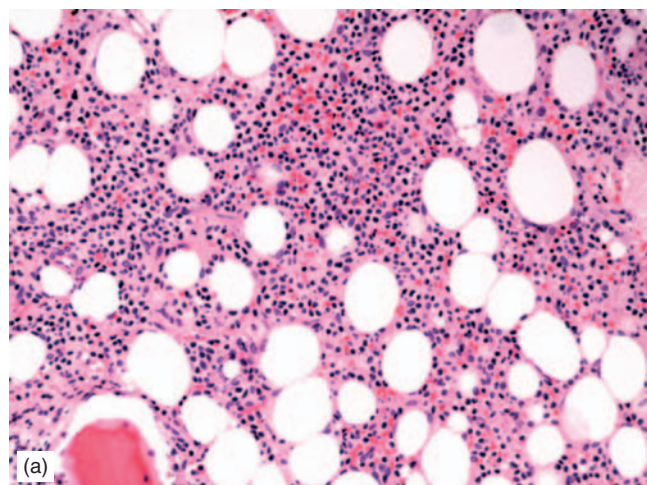


FIGURE 15.36 Hairy cell leukemia. Bone marrow biopsy section demonstrates an interstitial leukemic infiltrate (a). The tumor cells show nuclear spacing and a “fried egg” pattern (b). Bone marrow smear shows numerous tumor cells (c), some with cytoplasmic projections.

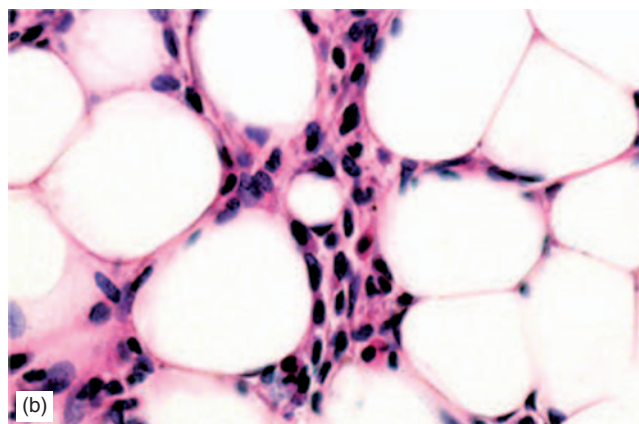
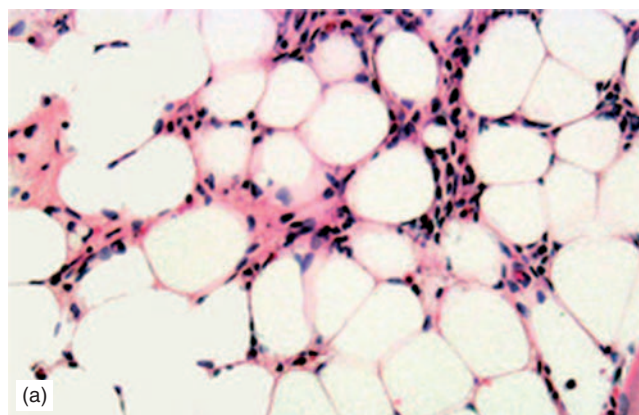


FIGURE 15.37 Hairy cell leukemia. The hypocellular variant of hairy cell leukemia may mimic aplastic anemia in bone marrow biopsy sections. Interstitial or focal hairy cell infiltration is sometimes overlooked (a and b). From Ref. [91] by permission.

The bone marrow biopsy sections often reveal moderate to marked increase in reticulin fibers leading to unsuccessful bone marrow aspiration (dry tap) (Figure 15.38). The reticulin fibers tend to surround the individual or small clusters of tumor cells. It has been shown that the hairy cells produce and assemble a fibronectin meshwork, which is in part responsible for the bone marrow fibrosis. In the vast majority of the cases, diagnosis of HCL is made by examination of bone marrow biopsy sections. In rare occasions, particularly when the bone marrow involvement is focal, the initial biopsy sections may not be diagnostic and deeper cuts or additional bone marrow biopsy is required.

Splenic involvement is one of the characteristic features of HCL, and splenomegaly is one of the most prominent clinical features. However, about 20% of the patients may lack significant splenomegaly. HCL infiltrates red pulp cords and sinusoids in a diffuse pattern (Figure 15.39). Scattered red blood cell lakes surrounded by hairy cells are often present. The white pulp is atrophic, and occasional small normal lymphoid aggregates may be present.

HCL involves the liver in up to 50% and the lymph nodes in about 15% of the cases. The hepatic infiltration is both portal and sinusoidal and the lymph node involvement is usually paracortical (Figure 15.40). Involvement of other tissues, such as skin and lung, is rare.

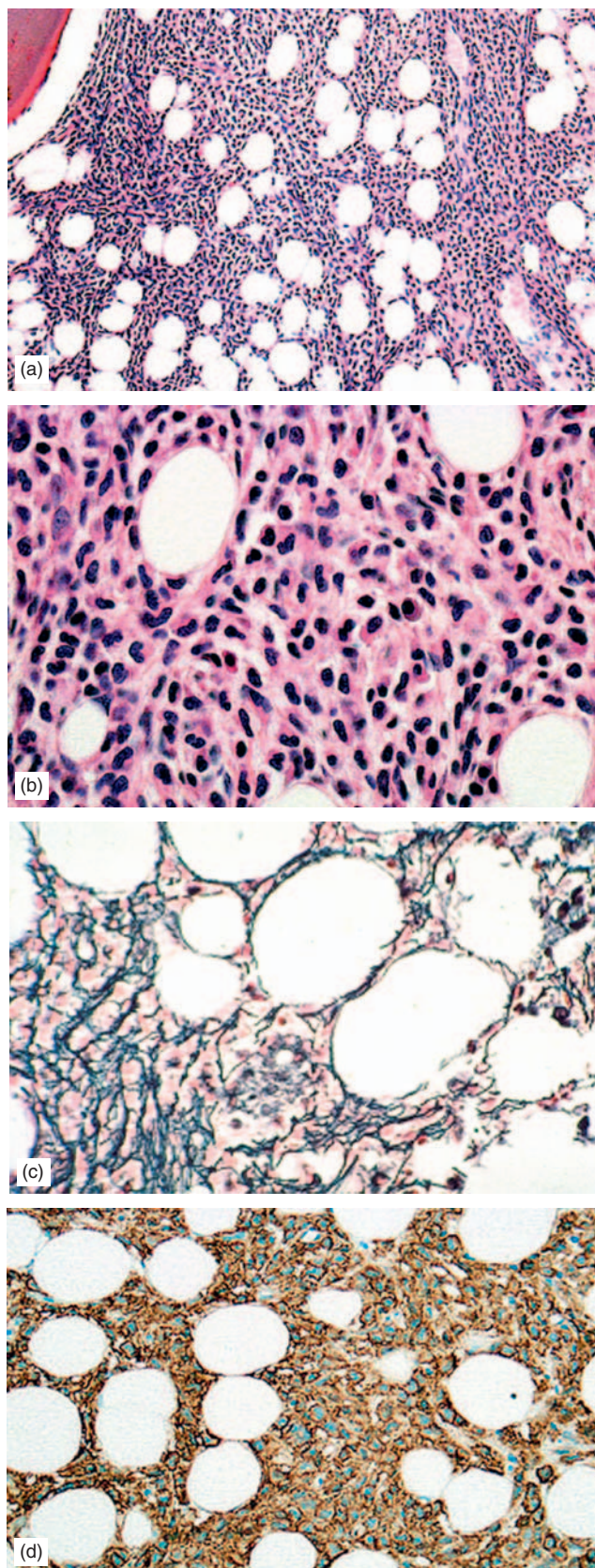


FIGURE 15.38 Hairy cell leukemia. Bone marrow biopsy section demonstrates an interstitial leukemic infiltrate: (a) low power and (b) high power. The reticulin stain shows increased reticulin fibers (c) and the tumor cells express DBA44 by immunohistochemical stain (d).

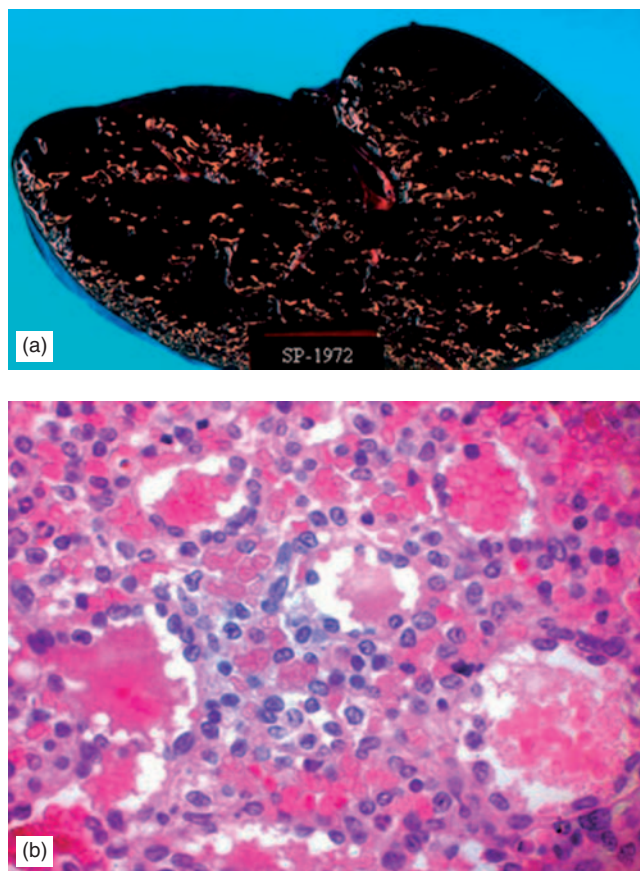


FIGURE 15.39 Splenic infiltration in hairy cell leukemia is diffuse (a) and involves the red pulp with the presence of red blood cell lakes (b).

Immunophenotype and Cytochemical Stains

Hairy cells are SIg⁺ (kappa or lambda) and express B-cell-associated markers, such as CD19, CD20, CD22, and CD79a. They characteristically coexpress CD25 and CD103 and are strongly positive for CD11c and FMC7 [150, 151]. The coexpression of CD25 and CD103 is rather unique for the HCL among the B-cell neoplasms (Figure 15.41). Immunohistochemical staining for DBA44 is also helpful in distinguishing hairy cells, though this antigen has been expressed by other lymphoid neoplasms [152]. Hairy cells are typically negative for CD5, CD10, CD23, CD27, and CD38 [153].

Hairy cells express an isoenzyme of acid phosphatase resistant to tartaric acid [141, 152]. Although the presence of TRAP is considered characteristic for hairy cells, occasionally cells in other lymphoid malignancies, such as Sezary syndrome, certain lymphomas, T-cell prolymphocytic leukemia, and Hodgkin lymphoma, may show TRAP positivity [141]. Gaucher cells may also express TRAP. Cytochemical TRAP stains have been routinely used for the diagnosis of HCL for many years, and now anti-TRAP antibodies are available for immunohistochemical studies.

None of the immunophenotypic and cytochemical markers are specific for HCL, but the expression of CD25, CD103, CD11c, FMC7, TRAP in a B-cell lymphoproliferative disorder is consistent with HCL if clinicopathologic findings are supportive of such diagnosis.

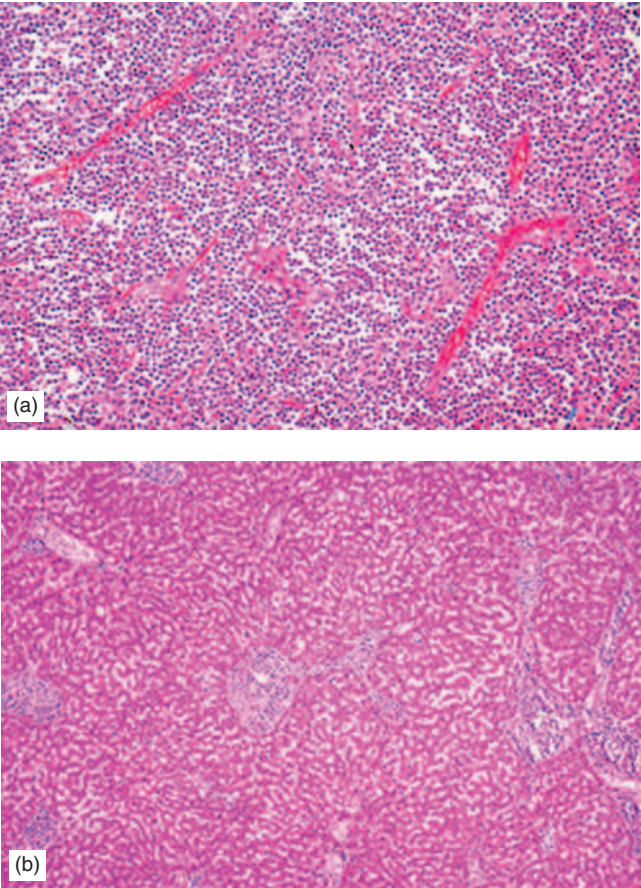


FIGURE 15.40 Hairy cell leukemia. (a) A lymph node biopsy section showing diffuse infiltration by hairy cells. (b) A liver biopsy showing infiltration of the portal areas and sinuses by the leukemic cells.

Cytogenetic and Molecular Studies

No specific cytogenetic or molecular aberrations have been found in HCL. Random chromosomal abnormalities such as 5q–, 6q–, 11q–, 17q– and trisomies 3, 4, 5, 12, and 18 have been reported [154–156]. Abnormalities of chromosome 5, most commonly trisomy 5 and interstitial deletions of band 5q13, have been observed in up to 40% of the cases [157].

As a disease of B-cells, HCL should show clonal immunoglobulin gene rearrangements [158]. The majority of HCL cases show mutation of the *VH* genes and express activation-induced cytidine deaminase, a molecule essential for somatic mutation and isotype switch [148]. Unlike most other B-cell neoplasms, HCL frequently expresses multiple Ig isotypes [159].

Clinical Aspects

HCL is relatively rare and accounts for about 2% of all lymphoid leukemias [1, 159]. The median age is about 55 years with a marked male predominance. Clinical symptoms are mostly related to splenomegaly and pancytopenia and include abdominal fullness or discomfort, fatigue,

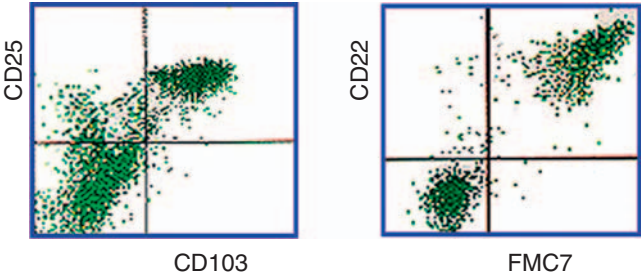


FIGURE 15.41 Hairy cell leukemia. Flow cytometry demonstrates expression of CD25, CD103, CD22, and FMC by the tumor cells. From Ref. [91] by permission.

TABLE 15.6 Clinical and laboratory findings in hairy cell leukemia.

Average age	55 years
Male:female ratio	3–5:1
Physical findings	
Splenomegaly	80%
Hepatomegaly	20–30%
Petechia and ecchymosis	30%
Lymphadenopathy	15–20%
Laboratory findings	
Anemia	85%
Thrombocytopenia	80%
Neutropenia	80%
Monocytopenia	80%
Hypergammaglobulinemia	20%

weight loss, fever, bruising, and bleeding [160–162]. Splenomegaly is present in about 80% of the HCL cases. Hepatomegaly is observed in 25–40% of the patients, but lymphadenopathy is not a major clinical feature and is found in 10–20% of the patients. Table 15.6 demonstrates a summary of the clinical and laboratory findings in HCL. Evidence of defective cell-mediated immunity has been demonstrated in HCL by several investigators [163–165].

Hairy cell leukemia is an indolent leukemia with an overall 12-year survival rate of >85% [166–168]. There are reports indicating that HCL patients without splenomegaly tend to remain free from significant neutropenia, have an excellent survival rate, and are usually older than the patients with splenomegaly. There is also some evidence that the morphology of the hairy cells may correlate with prognosis. For example, there are reports indicating that the HCL cells with oval nuclei are associated with better prognosis than the ones with convoluted or indented nuclei and that the nuclear convolution is more frequently associated with marrow fibrosis and severe pancytopenia [169, 170]. Therapeutic modalities include interferon alpha, purine analogs such as pentostatin (2'-DCF) and cladribine (2-CdA), splenectomy, and rituximab (anti-CD20) therapy [171–173].

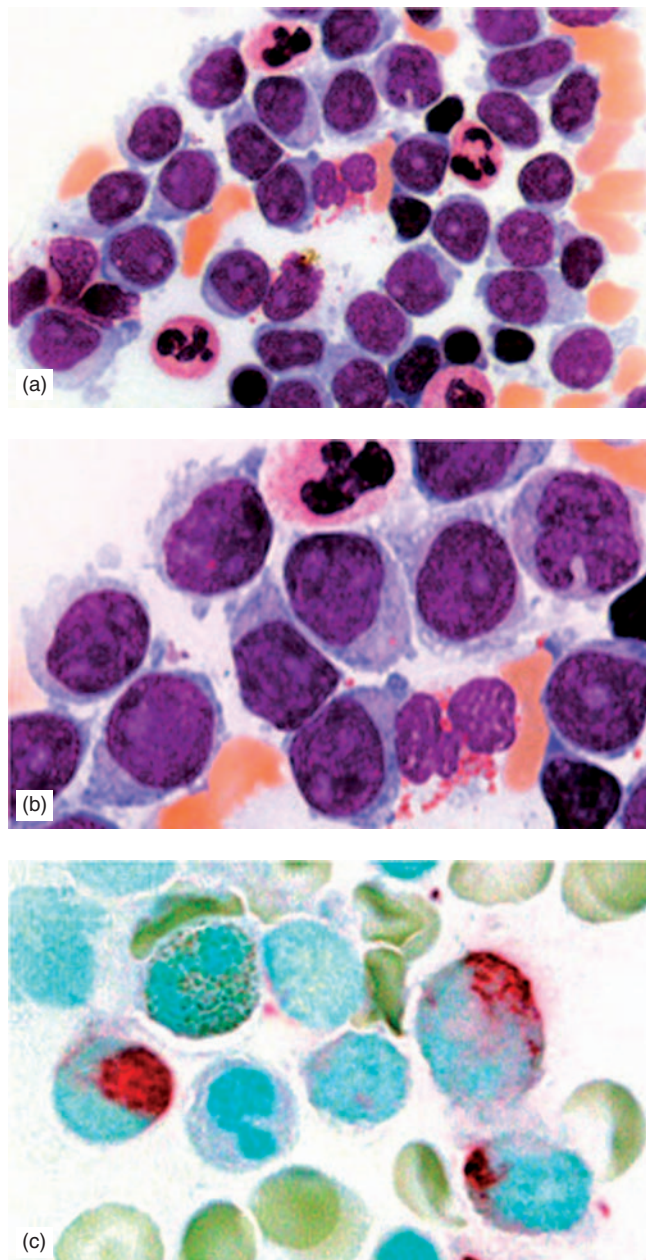


FIGURE 15.42 Hairy cell leukemia, morphologic variant. Bone marrow smear (a: low power; b: high power views) shows polymphocyte-like hairy cells. These cells are TRAP-positive (c). From Ref. [91] by permission.

Hairy Cell Leukemia Variants

Rare cases of HCL show morphologic features intermediate between those of hairy cells and polymphocytes (Figure 15.42). This variant is characterized by elevated white blood cell count of usually $>50,000/\text{mL}$ and lack monocytopenia. The neoplastic cells have abundant basophilic cytoplasm with cytoplasmic projections, moderately dense heterochromatin, and a prominent nucleolus. These cells, in contrast to typical HCL cells, lack the expression of CD25 and may also be negative for CD103 and/or TRAP [174, 175].

An unusual, rare morphologic variant of HCL has been reported in which the tumor cells have multilobated nuclei [176]. A blastic variant of HCL has been described in which the blast cells are TRAP-positive and show fine cytoplasmic projections [177].

Differential Diagnosis

The differential diagnosis of HCL includes SMZL, PLL, and atypical CLL/SLL. HCL involves the splenic red pulp in a diffuse pattern with atrophy of the white pulp, whereas SMZL involves the white pulp and often has a nodular pattern. Bone marrow fibrosis and interstitial infiltration are common features of HCL, whereas in SMZL bone marrow fibrosis is infrequent and intrasinusoidal infiltration is the characteristic feature. Unlike SMZL cells, HCL cells are TRAP-positive and express CD25 and CD103 (see Table 15.6).

Hairy cell variant with polymphocytic features is distinguished from PLL and atypical CLL (CLL/PLL) by the coexpression of CD25 and CD103 and TRAP positivity. CLL/PLL cells often express CD5 and CD23 and have different cytogenetic profiles.

FOLLICULAR LYMPHOMA

Follicular lymphoma is the second most common lymphoma and represents a neoplasm of follicle center B-cells consisting of a mixture of centrocytes and centroblasts [1, 2, 93]. The pattern of lymph node involvement is at least partially follicular. Other terminologies used for this lesion are “follicle center lymphoma” and “follicular center cell lymphoma.”

Etiology and Pathogenesis

The etiology of FL is not known. Inhibition of apoptosis as the result of overexpression of BCL-2 appears to play a critical role in the pathogenesis of this disorder [178–180]. Approximately 75–90% of patients with FL demonstrate $t(14;18)(q32;q21)$ resulting in the juxtaposition of the *BCL-2* gene on chromosome 18 into the *IGH* heavy chain locus on chromosome 14. This translocation leads to constitutive expression of BCL-2 giving the transformed cells an extended survival and growth advantage. The $t(14;18)$ alone, however, does not seem to be sufficient for the development of lymphoma. It keeps the transformed cells alive and therefore at risk for subsequent cytogenetic alterations necessary for the development of a fully malignant phenotype to occur [178].

Published studies demonstrate methylation of androgen receptor, SHP1, and death-associated protein kinase genes in FL [181]. By contrast, methylation of the cyclin-dependent kinase inhibitors p15, p16, and p57 is uncommon in FL and may indicate an important step toward the transformation of FL to a more aggressive lymphoma.

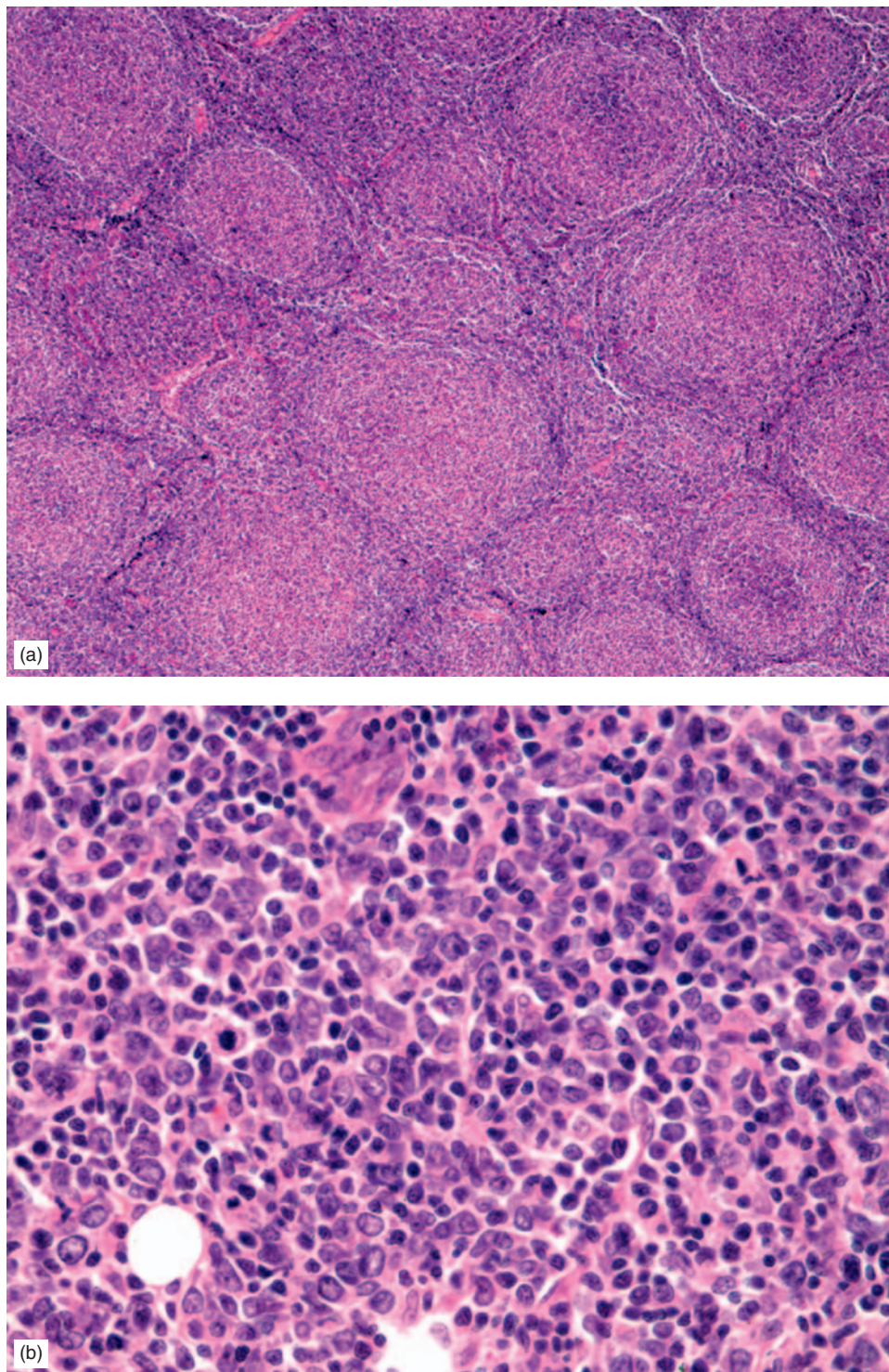


FIGURE 15.43 Follicular lymphoma; lymph node section. (a) Low power view demonstrating back-to-back follicles of various sizes and remnants of mantle zones. (b) High power view showing a mixed population of centrocytes and centroblasts.

Pathology

Morphology

The involved lymph nodes show effacement of the nodal architecture with a lymphoproliferative process which displays a predominantly follicular pattern (Figures 15.43 and 15.44) [1, 2, 93]. The neoplastic follicles are densely packed against one another, are often ill-defined, and show loss

of or minimal mantle zone areas. These follicles show no polarity or tingible body macrophages and consist of various proportions of centrocytes and centroblasts interspersed with T-cells and follicular dendritic cells. Centrocytes are small to medium sized, show scant pale cytoplasm, irregular (angulated, twisted, convoluted) nucleus, and inconspicuous nucleoli [1]. Centrocytes are the predominant cells in the majority of the cases. Centroblasts are large transformed

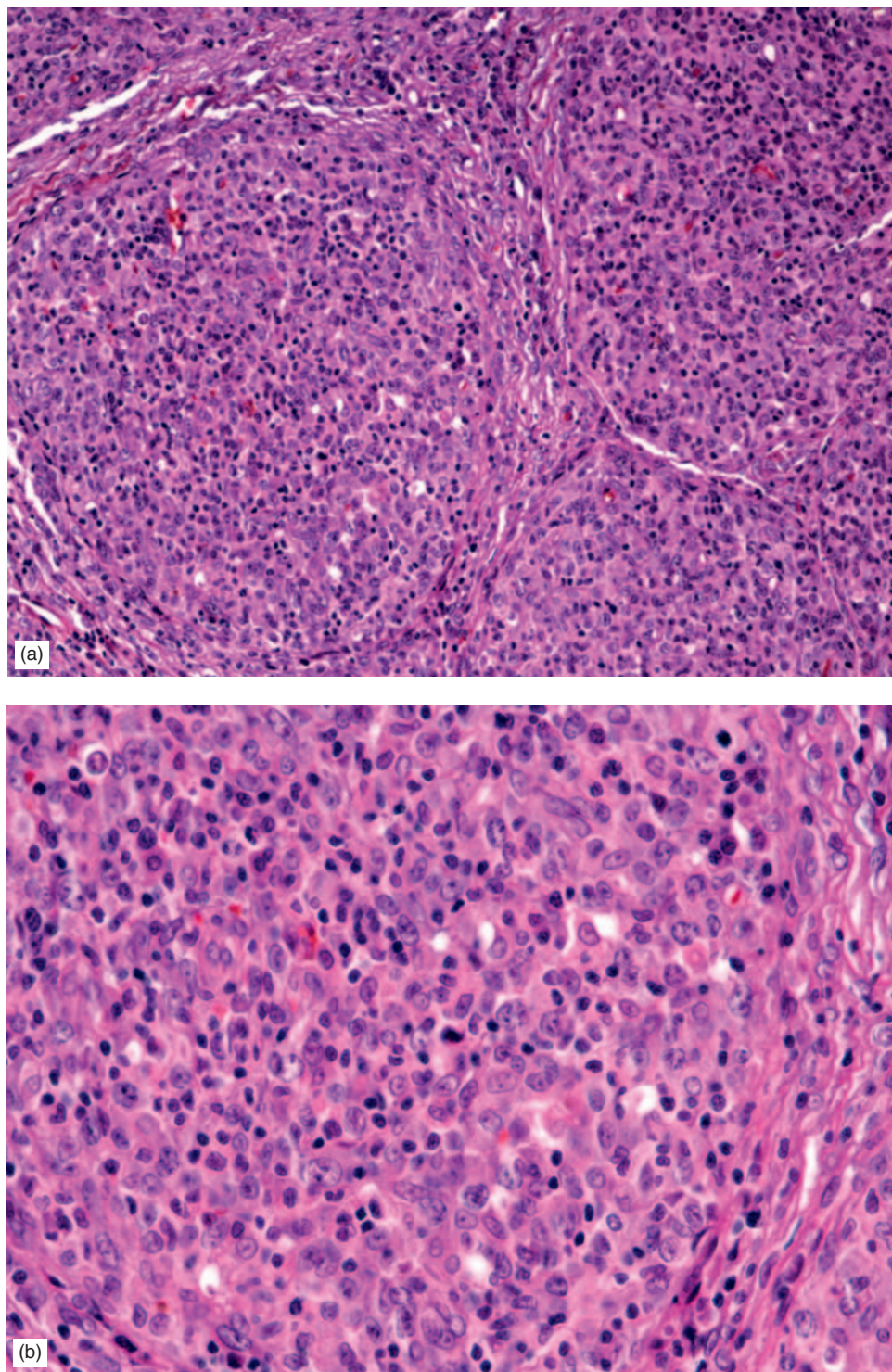


FIGURE 15.44 Follicular lymphoma; lymph node section. (a) Intermediate power view demonstrating back-to-back follicles with loss of mantle zones. (b) High power view showing a mixed population of centrocytes and centroblasts.

cells with small amounts of basophilic cytoplasm, round, oval, or slightly irregular nuclei, vesicular chromatin, and one to three nucleoli located close to the nuclear membrane. In some cases, centroblasts may show significant atypical features, such as hyperchromatic or markedly irregular nuclei. Follicular dendritic cells are also large and have a round nucleus and a vesicular chromatin, but unlike centroblasts, they often appear in doublets or show double nuclei,

their nucleoli are centrally located, and they have ill-defined pale cytoplasm. These cells express CD21 and CD23.

Areas of diffuse involvement may be present, often associated with fibrosis. The neoplastic cells are usually present in the interfollicular areas and are easily identified by immunohistochemical stains (CD10+, BCL-6+). Discrete clusters of marginal zone monocytoid B-cells may be present in about 10% of the cases [1]. The pattern of

involvement is divided into three categories:

1. Follicular: >75% of the involved tissue shows follicular pattern.
2. Follicular and diffuse: 25–75% of the involved tissue shows follicular pattern.
3. Minimally follicular: <25% of the involved tissue shows follicular pattern.

The following grading system has been recommended for FLs based on the proportion of centroblasts per 40× high-power microscopic field (hpf):

Grade 1	0–5 centroblasts/hpf
Grade 2	6–15 centroblasts/hpf
Grade 3	>15 centroblasts/hpf
3a	Centrocytes present
3b	Solid sheets of centroblasts

According to the WHO recommendations, variations in the pattern or grading observed in different areas of the involved tissue should be mentioned in the pathology report.

Bone marrow involvement is observed in 40–45% of the cases and is typically paratrabecular (Figures 15.45 and 15.46) [1, 2, 93, 182]. The lymphomatous aggregates usually do not show follicular configuration and consist of a mixture of centrocytes and centroblasts. There may be discordance between morphologic findings of the involved bone marrow and the lymph node in the same patient. In such cases, the grade of bone marrow involvement is often less than that of the involved lymph node. For example, in a patient with a grade 3 FL in the lymph node, the bone marrow involvement may appear as grade 1 or 2. Peripheral blood involvement is a frequent finding with the presence of atypical lymphoid cells with nuclear clefts or notches (Figure 15.46b).

Immunophenotype

The neoplastic cells of FL express B-cell-associated molecules, such as CD19, CD20, CD22, and CD79a. They are positive for CD10, cytoplasmic BCL-2, and nuclear BCL-6 in the majority of the cases and typically negative for CD5, CD11c, and CD43 (Figures 15.47 and 15.48) [1, 2, 182–184]. Expression of CD23 is variable and the Ki-67 fraction is low. A tight meshwork of CD21⁺ and CD23⁺ cells is present in the neoplastic follicles representing follicular dendritic cells.

The higher grade FLs may lack the expression of BCL-2 but may express CD43 [1, 2].

Cytogenetic and Molecular Studies

Cytogenetic abnormality is a common feature in FL. The most common chromosomal translocation in this disorder is t(14;18)(q32;q21), which has been observed in approximately 85% of the cases (Figure 15.49) [178]. This translocation places *BCL-2* gene on chromosome 18 next to the *IGH* heavy chain locus on chromosome 14 [178]. The resultant overexpression of BCL-2 confers a survival advantage on

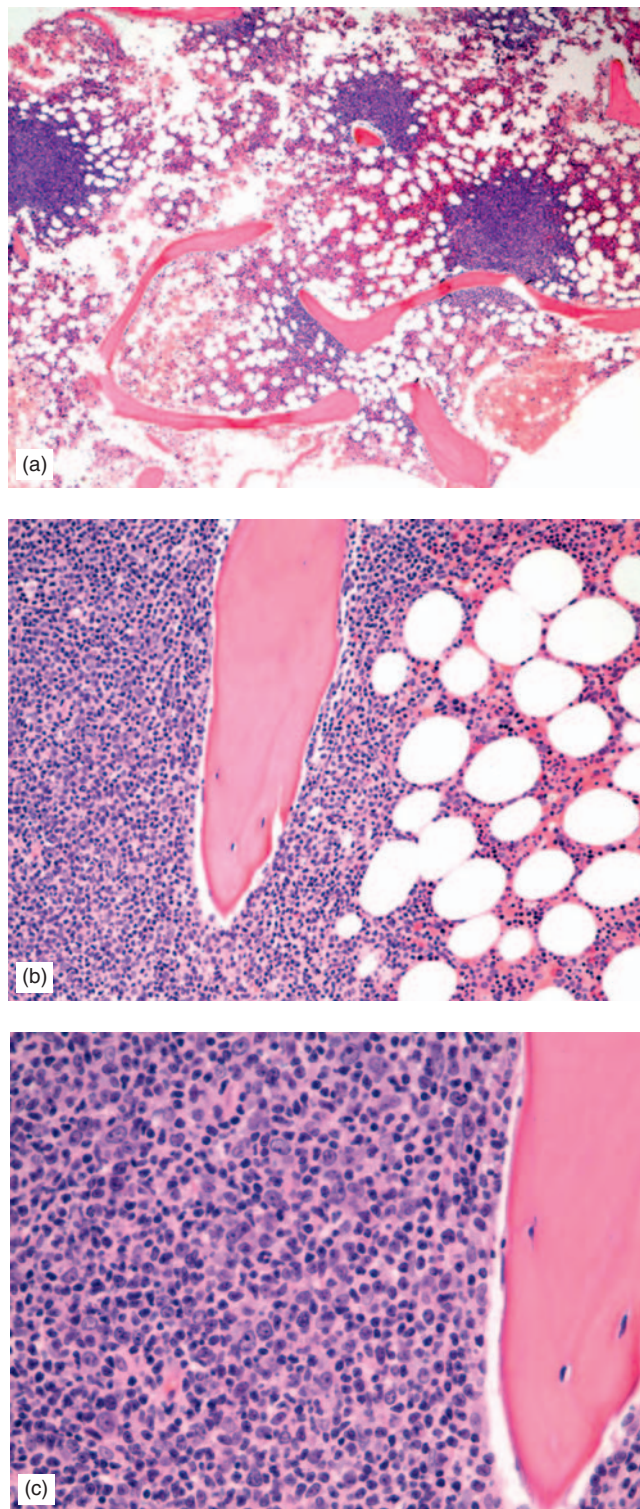


FIGURE 15.45 Follicular lymphoma. Bone marrow section showing paratrabecular lymphoid infiltrates: (a) low power, (b) intermediate power, and (c) high power views.

these B-cells, which now defies apoptosis. The continuing accumulation of these cells, rather than their rapid proliferation, is the hallmark of this low-grade lymphoma. In some patients, a subsequent genetic event involving a gene for cell

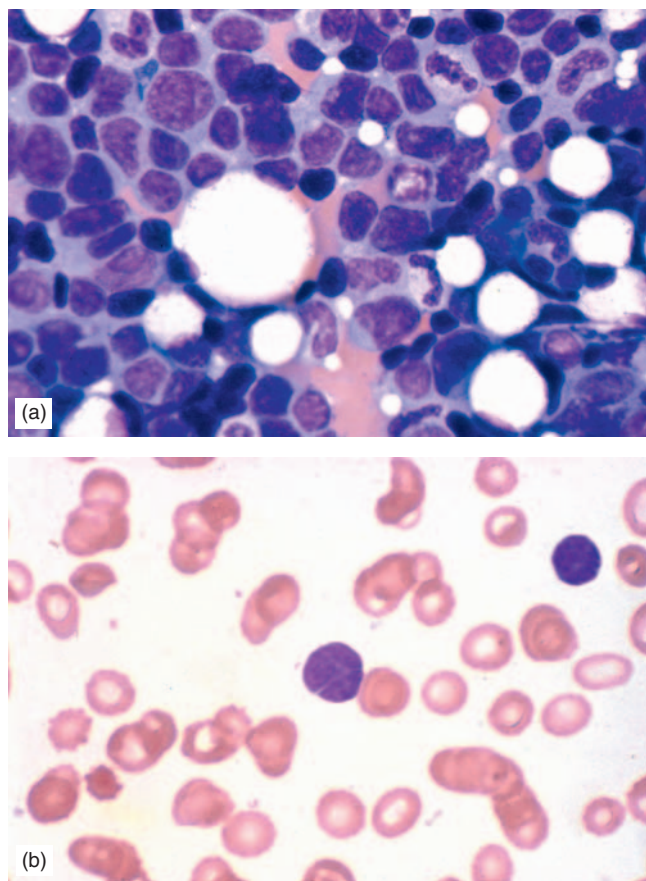


FIGURE 15.46 Bone marrow (a) and peripheral blood smears (b) of a patient with follicular lymphoma demonstrating atypical lymphocytes with irregular or cleaved nuclei.

proliferation (e.g. *ras*) may transform indolent disease into a more aggressive lymphoma. The *BCL-2* protein is not normally expressed in germinal center cells. Variant translocations have been described, $t(2;18)(p12;q21)$ and $t(18;22)(q21;q11.2)$, involving the *IGK* or *IGL* genes, respectively, rather than *IGH*. There have also been complex translocations described involving a third chromosome breakpoint in addition to 14q32 and 18q21 [185].

In addition to the G-banding techniques, this translocation is detected by molecular methods using Southern blot, qualitative PCR testing, or quantitative (real-time) PCR (Figure 15.50). Recent studies have shown prognostic correlation with the level of *BCL-2* fusion genes using quantitative PCR methods [186, 187]. Most cases demonstrate a pattern of translocation involving the major breakpoint region (MBR) of the *BCL-2* gene with a smaller proportion involving the minor cluster region (MCR). Primers specific for MBR and MCR are required for detection of each. And about 25% of cases involve other breakpoints that will not be detected by either primer set and require additional custom primers [188]. That is one reason why the cytogenetic test for $t(14;18)$ will actually pick up more cases than the molecular test [189].

The presence of $t(14;18)$ is not specific for FL and has been found in 15–20% of diffuse large B-cell and MALT lymphomas. Most FL patients show, in addition to $t(14;18)$, clonal evolution events leading to additional chromosomal

aberrations such as +8, +7, +12, +18 or abnormalities of 3q, 6q, 13q, and 17q (Table 15.7) [1, 190–193]. The 3q27 abnormality involves the *BCL-6* gene and is present in about 15% of the cases. A more aggressive variant of FL (grade 3b) is less frequently associated with $t(14;18)$, but often carries the $t(3;14)$ involving the *BCL-6* oncogene. The 17p13 abnormalities involve the *p53* gene is often associated with the transformation of FL [1]. In addition, gene expression profiling is beginning to be applied to FL (Figure 15.51) [194].

Clinical Aspects

FL is the second most common lymphoma comprising between 22% and 35% of the non-Hodgkin lymphomas in the Western Europe and United States, respectively. It is the most common indolent lymphoma in Western countries accounting for up to 70% of all low-grade lymphomas [2].

The median age at diagnosis is about 60 years with slight female predominance. Painless peripheral lymphadenopathies in the cervical, axillary, and inguinal regions are the major clinical presentations. Mediastinal and hilar lymph nodes, bone marrow, spleen, and liver are also frequently involved. Involvement of the central nervous system is rare. Systemic “B” symptoms are present in about 20% of the cases and patients may rarely present primary extranodal disease involving skin or other tissues. The clinical course is variable and primarily depends on the stage and grade of the disease. The median survival is 7–10 years for patients with grade 1 or 2 and stage III to IV diseases [2].

An international prognostic index (IPI) has been proposed for FL, which includes the following five adverse prognostic factors [195, 196]:

1. Age >60 years
2. Stage III or IV
3. Hemoglobin level <12 g/dL
4. Number of involved nodal areas >4
5. Elevated serum lactate dehydrogenase (LDH).

Patients with FL fall into three major groups: low risk with 0 to 1 adverse factors (36%), intermediate risk with 2 adverse factors (37%), and high risk with 3 or more adverse factors (27%) [195–197].

Therapeutic modalities range from involved field radiotherapy for stages I and II to combination chemotherapy for advanced stages [198]. Rituximab has also been added to the therapeutic regimens, particularly in relapsed or refractory lymphomas [199]. Autologous and allogeneic stem cell transplantations have been attempted for patients with recurrent FL, though limitations such as high recurrence rate and risk of secondary MDS still exist [200]. The anti-idiotypic vaccination in FL is under evaluation [178].

Variants of Follicular Lymphoma

Two major variants of FL are recognized: primary cutaneous follicle center cell lymphoma and diffuse follicle center cell lymphoma.

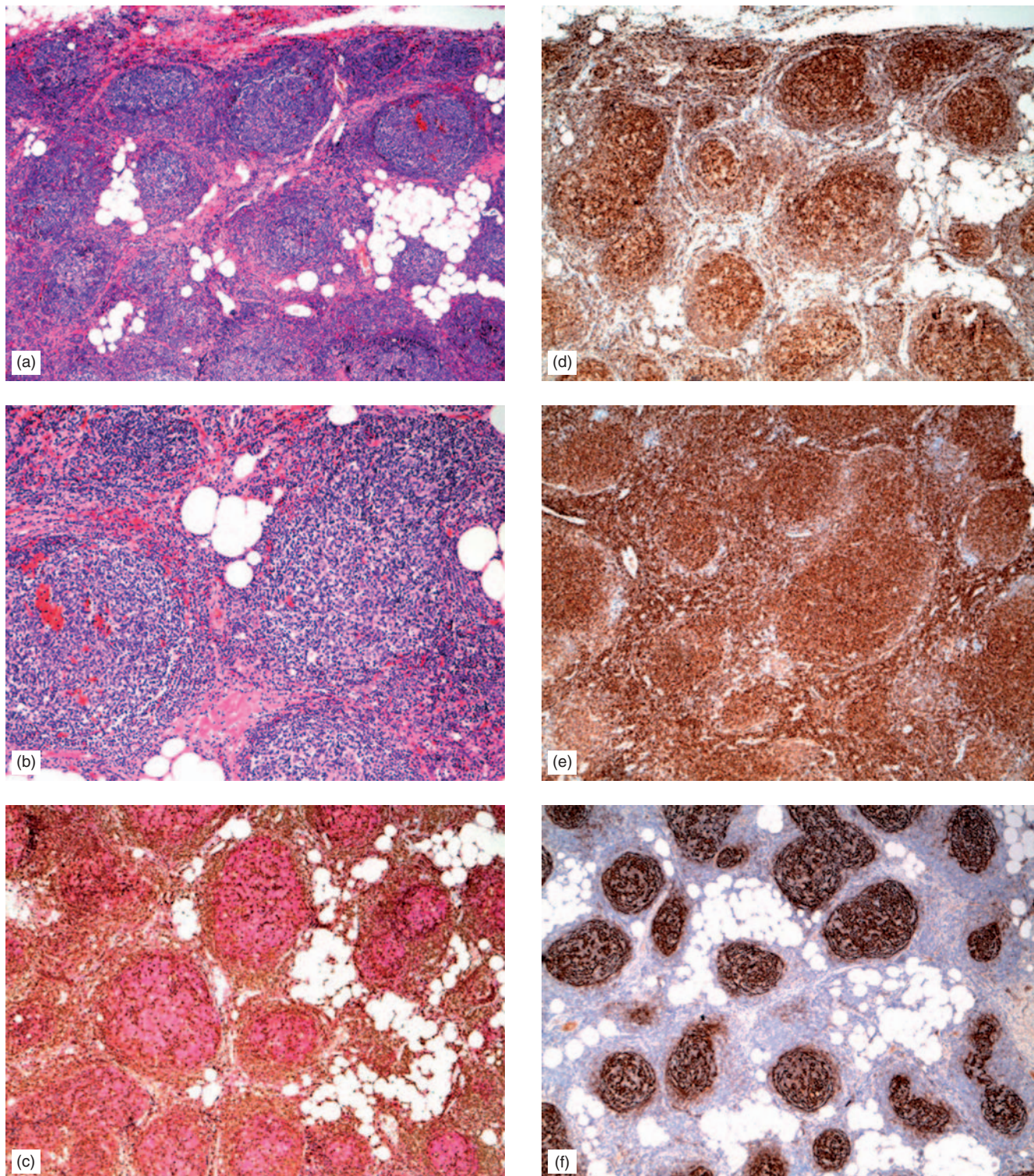


FIGURE 15.47 Follicular lymphoma; lymph node section. (a) Follicular structures are separated from one another with a rim of mantle cells resembling reactive follicles. (b) Higher power view demonstrates lack of tingible body macrophages and some degree of fibrosis. (c) Dual immunohistochemical stains for CD3 (brown) and CD20 (red) show predominance of CD20⁺ cells within the follicles. These cells are positive for CD10 and BCL-2 (d and e, respectively). CD21 stain shows a meshwork of interfollicular dendritic cells (f).

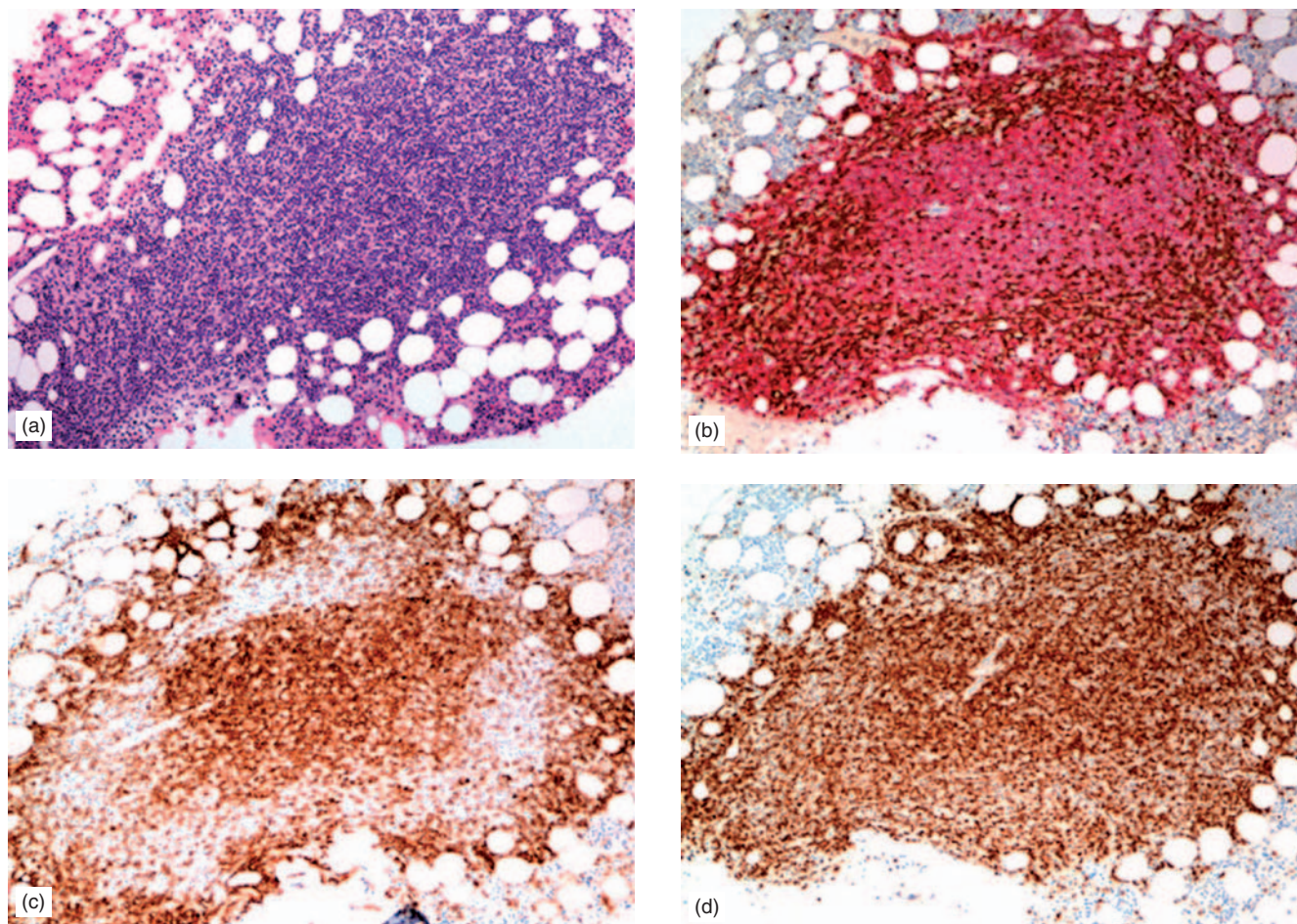


FIGURE 15.48 Bone marrow lymphomatous involvement. (a) A lymphoid aggregate with expansion into the surrounding fatty tissue. (b) Dual immunohistochemical stains for CD3 (brown) and CD20 (red) show CD20+ cells in the center surrounded with CD3+ cells. The CD20+ cells also express CD10 (c) and bcl-2 (d).

Primary cutaneous follicle center cell lymphoma is a B-cell cutaneous lymphoid malignancy usually occurring on the head, neck, and trunk. The lesions tend to be limited to skin without nodal involvement. The lymphomatous infiltrate consists of a mixture of centrocytes and centroblasts and appear partially follicular. The tumor cells are often positive for CD10 and BCL-6 and negative for BCL-2 [201]. Approximately 6% of the cases demonstrate t(14;18) and 6% show extracutaneous progression [202, 203].

Diffuse follicle center lymphoma is a rare lymphoma primarily consisting of centrocytes with a diffuse infiltrating pattern [1]. It is divided into two grades based on the percent of centroblasts per high-power microscopic field (hpf): grade 1 with 0–5 centroblasts/hpf and grade 2 with 6–15 centroblasts/hpf. The neoplastic cells show immunophenotypic features similar to the FL, including expression of CD10, bcl-2, and bcl-6.

Differential Diagnosis

The major differential diagnosis is between FL and reactive follicular hyperplasia (RFH). Reactive follicles are usually

separated by interfollicular areas, are surrounded by a mantle zone, show polarity, contain tingible body macrophages, and lack BCL-2 expression. Neoplastic follicles are often back to back or merging, show minimal or no mantle zone, appear monomorphic with loss of polarity, lack tingible body macrophages, and express BCL-2 (Table 15.8). There is evidence of interfollicular infiltration by the presence of CD10 and BCL-6 positive cells in these areas.

Follicular colonization in MALT lymphomas may mimic FL. However, the clinical setting and immunophenotypic features of MALT lymphoma (CD10–, BCL-6–) are different from those of FL (CD10+, BCL-6+). Also, MCLs with nodular or follicular patterns may simulate FL (see the following section).

MANTLE CELL LYMPHOMA

Mantle cell lymphoma (MCL) is an aggressive B-cell neoplasm consisting of small, mature centrocyte-like cells with

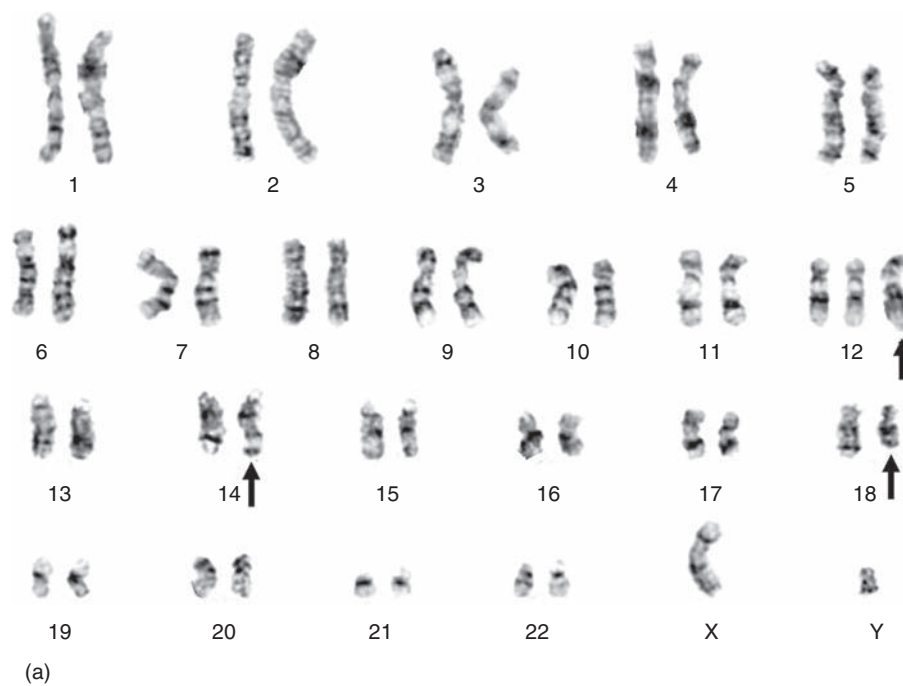
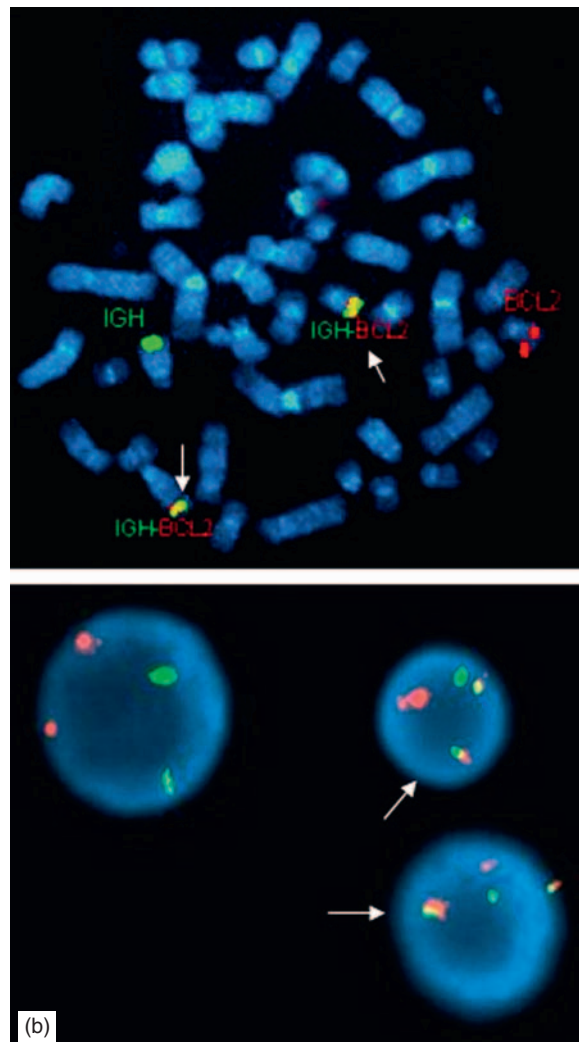


FIGURE 15.49 Karyotype (a) and FISH (b) analysis of lymphoma cells from a patient with follicular lymphoma demonstrating 47,XY, +12,t(14;18)(q32;q21) and *IGH-BCL-2* fusion.



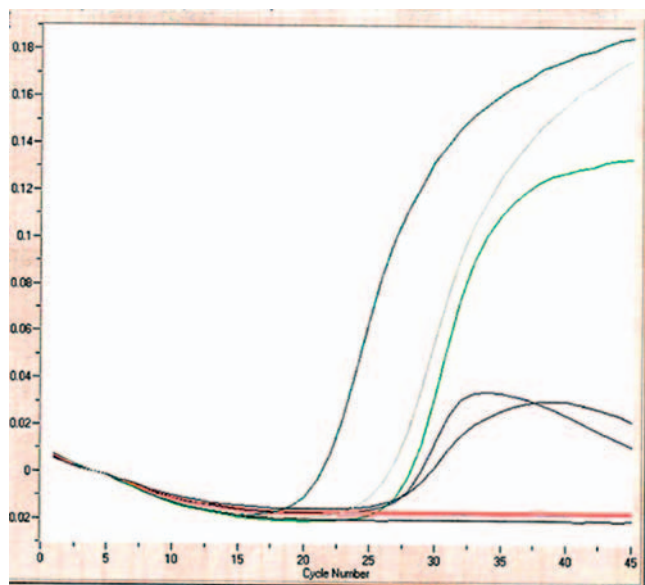


FIGURE 15.50 *BCL-2* fusion gene quantitation by real-time PCR. Patient 1 is positive for *BCL-2*, demonstrating amplification of the target with mid-log point at about cycle 30 (blue curve). The tan curve is the amplification of the control reference gene in the specimen, tPA. Patient 2 is negative for *BCL-2*, demonstrating no amplification of this target (red curve). The tan curve represents the tPA control target for Patient 2, confirming that there are no inhibitors of PCR in the specimen.

TABLE 15.7 Cytogenetic abnormalities in follicular lymphoma.*

Abnormalities	Frequency (%)
Structural	
t(14;18)(q32;q21)	78
3q27-28	16
17p**	15
del(6q)	13
1q12-21	13
1p21-22	10
10q22-24	10
Numerical	
+X	21
+7	20
+18	20
+12/dup(12q)	10

*Adapted from Tilly H, Rossi A, Stamatoullas A, Lenormand B, Bigorgne C, Kunlin A, Monconduit M, Bastard C. (1994). Prognostic value of chromosomal abnormalities in follicular lymphoma. *Blood* **84**, 1043–9.

**Associated with a worse prognosis.

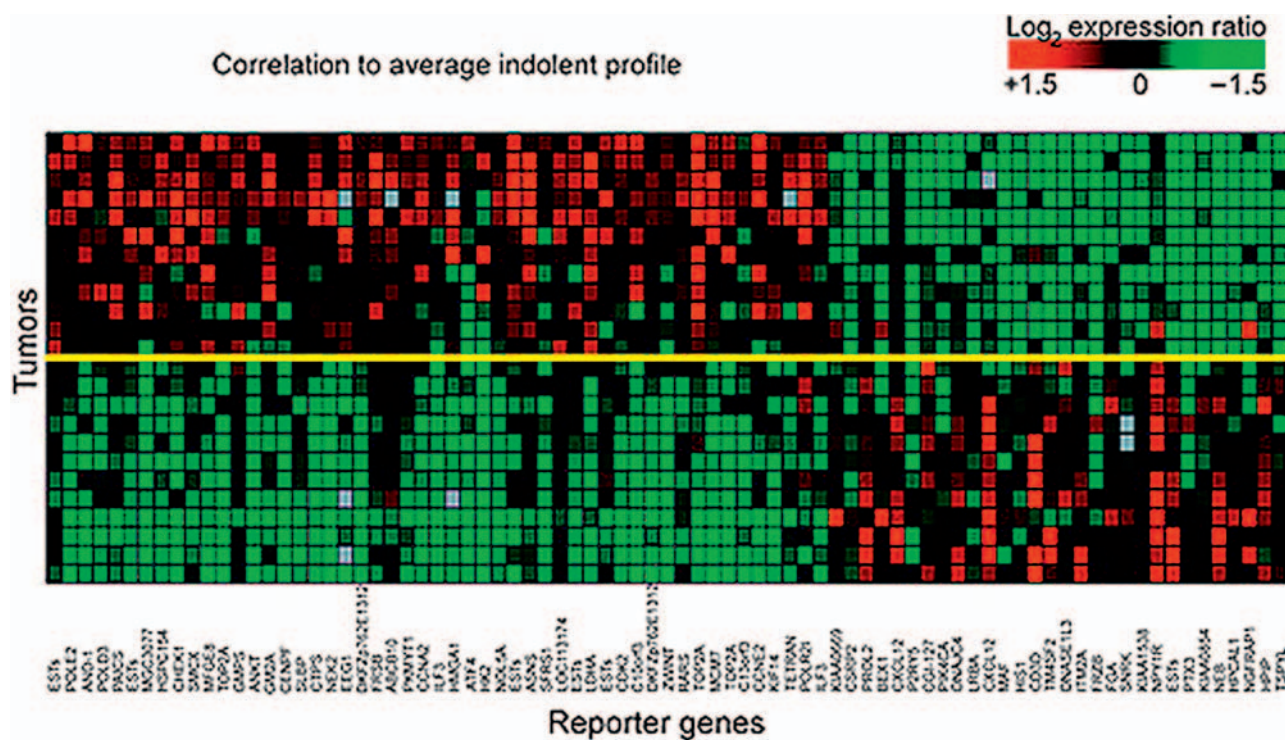


FIGURE 15.51 Gene expression profiling in follicular lymphomas using a microarray technique. Results demonstrate two populations of patients: those with aggressive disease are segregated above the solid yellow line and those with indolent clinical course are placed below it. From Ref. [194] by permission. This research was originally published in *Blood*.

TABLE 15.8 Morphologic features distinguishing follicular lymphoma (FL) from reactive follicular hyperplasia (RFH).

Features	FL	RFH
Follicles	Back to back or merging, often with loss of mantle zone Lack of polarity Lack of tingible body macrophages Monoclonal Commonly BCL-2 positive Low Ki-67 fraction	Separated, with preservation of mantle zone Presence of polarity Presence of tingible body macrophages Polyclonal BCL-2 negative High Ki-67 fraction
Interfollicular areas	CD10 positive cells present BCL-6 positive cells present	CD10 positive cells absent BCL-6 positive cells absent

scant cytoplasm and slightly irregular nuclei. MCL lacks centroblasts and immunoblasts [1, 2, 204, 205].

Etiology and Pathogenesis

The etiology of MCL is not known. The *BCL-1* (*cyclin D1*, abbreviated *CCND1*) gene located on the chromosome 11q13 apparently plays an important role in the pathogenesis of MCL [205, 206]. *BCL-1* is an important regulator of the G1 phase of the cell cycle. The chromosomal translocation t(11;14)(q13;q32) leads to the upregulation of *BCL-1* leading to the inhibition of the suppressive effect of Rb and p27, amplification of the cyclin-dependent kinase-4 (CDK-4), deletion of CDK inhibitor p16, and overexpression of p16 transcriptional repressor BMI-1, causing dysregulation of the cell cycle [205–207].

Pathology

Morphology

The affected lymph node shows effacement of the nodal architecture with an infiltrative process with a histologic pattern that may be diffuse, vaguely nodular, marginal zone pattern, or a combination of these (Figures 15.52–15.54) [1, 2, 208]. Most commonly, the involved lymph node shows transitional areas between diffuse and nodular patterns, but occasionally nodular pattern is predominant. In the marginal zone pattern, the neoplastic cells expand the mantle zone area surrounding a germinal center [205]. This pattern is more common in spleen. A prominent meshwork of follicular dendritic cells is usually present.

The cytologic features in typical MCL cases consist of monotonous small to medium-sized lymphocytes with scant cytoplasm, slightly irregular nucleus, condensed chromatin, and inconspicuous nucleoli (Figure 15.52). Hyalinized small vascular structures are frequently present. Centroblasts and immunoblasts are typically absent, though centroblasts may be present in remnants of germinal centers. In some cases, a proportion of the neoplastic cells may show more abundant cytoplasm and appear like monocytoid

B-cells. The neoplastic cells in occasional cases may mimic CLL/SLL with small lymphocytes, round nucleus, and condensed chromatin. A prolymphocyte-like variant with marked leukocytosis mimicking PLL has also been described. These patients usually have splenomegaly and demonstrate clinicopathological features very similar to PLL, except for the demonstration of t(11;14).

The blastoid variant of MCL is usually referred to the cases in which the neoplastic cells resemble lymphoblasts, with dispersed chromatin, prominent nucleoli, and high mitotic figures (Figure 15.55) [208–210]. A pleomorphic type consisting of large cells with oval or irregular nuclei has also been reported [1, 211]. A summary of the cytologic variants of MCL is presented in Table 15.9.

Extranodal involvement is frequent in MCL and often involves bone marrow, peripheral blood, spleen, liver, and gastrointestinal tract [212–215]. Bone marrow infiltration is reported in 50–80% of the cases. The pattern of involvement is often a combination of nodular, interstitial, and paratrabeular. Isolated paratrabeular infiltrations are rare. The neoplastic cells may also be present in the peripheral blood in various numbers creating a condition mimicking CLL. The spleen is affected in 30–50% of the cases with the primary involvement of the white pulp. The white pulp nodules are expanded and often are merged, sometimes surrounding the residual germinal centers. The extent of splenic red pulp involvement is variable. The most common clinical presentation of gastrointestinal involvement in MCL is *lymphomatous polyposis* with the presence of multiple intestinal lymphoid polyps [216].

Immunophenotype

The MCL cells express B-cell-associated markers such as CD19, CD20, CD22, and CD79a with surface IgM⁺ and IgD⁺. The Ig lambda light chain restriction is observed more often than the kappa light chain. The neoplastic cells are also positive for CD5, FMC7, CD43, BCL-1, and BCL-2, and lack of the expression of CD10, BCL-6, and often CD23 [1, 207, 217–219]. In some cases, CD23 is weakly expressed. The combination of BCL-1 and CD5 expression is characteristic immunophenotypic feature of MCL (Figure 15.54). The Ki-67 labeling is typically low,

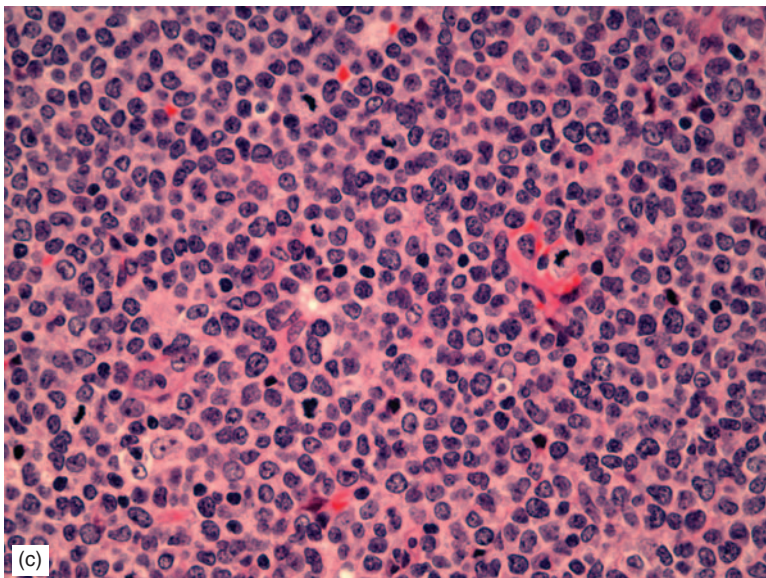
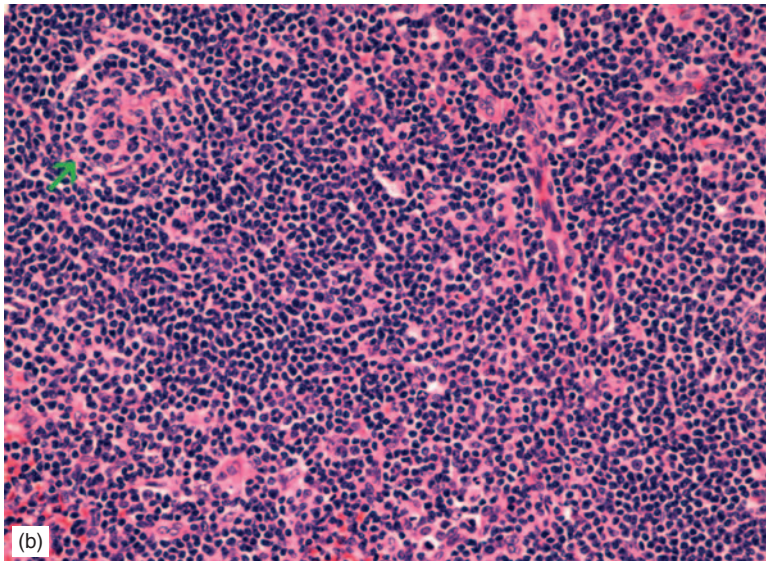
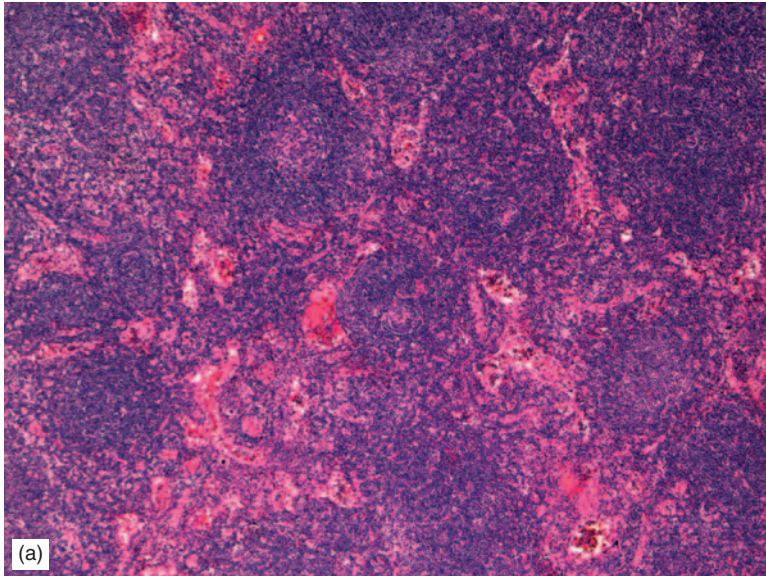


FIGURE 15.52 Mantle cell lymphoma. Lymph node section demonstrates expansion of mantle zones with remnants of follicular structures: (a) low power and (b) intermediate power views. Tumor cells are monomorphic and show some mitotic figures: (c) high power view.

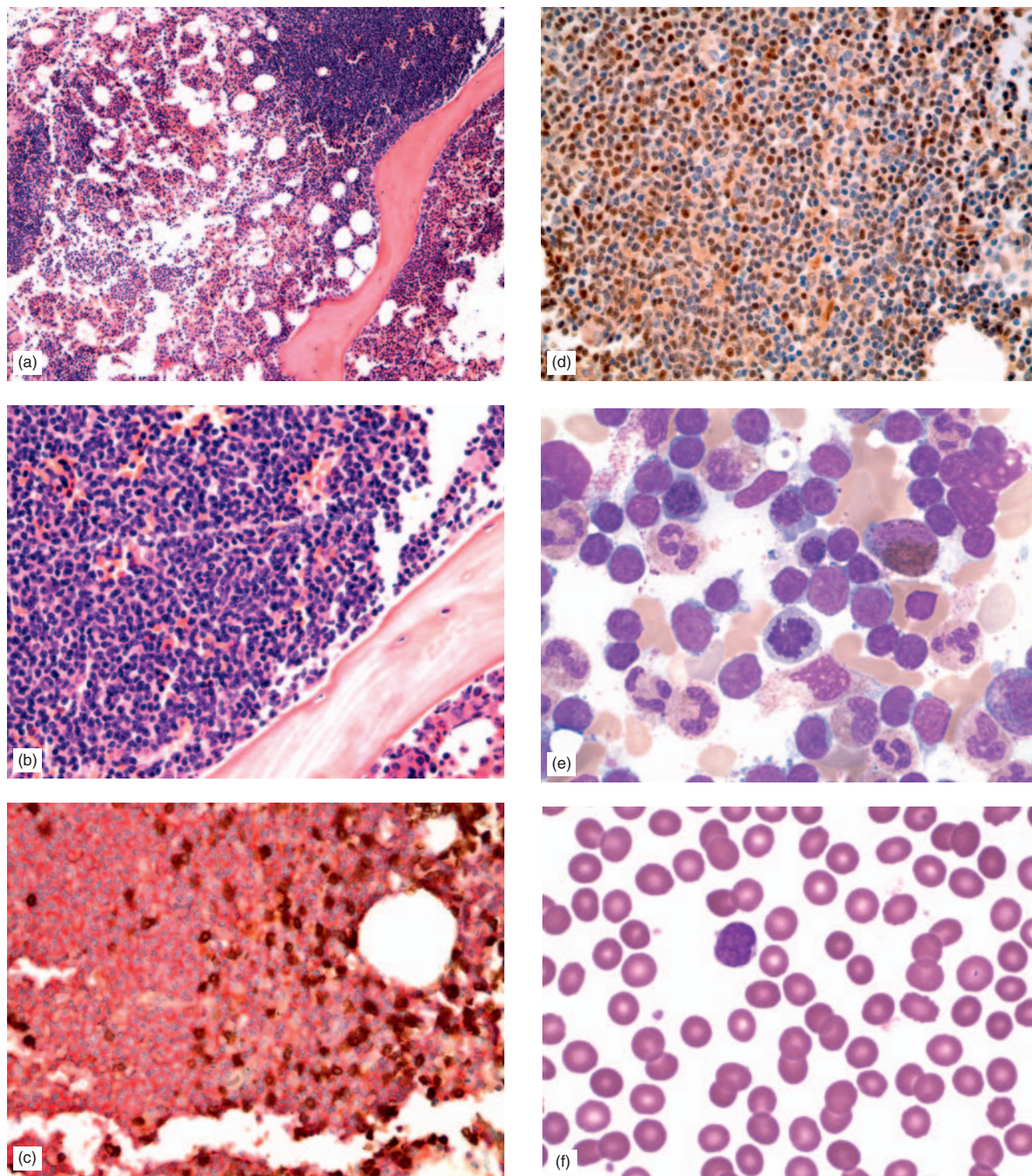


FIGURE 15.53 Bone marrow biopsy section demonstrating involvement with mantle cell lymphoma. Immunohistochemical stains show tumor cells (a: low power, and b: high power) expressing CD20 (c, red) and BCL-1 (d). Bone marrow smear shows numerous atypical lymphoid cells (e) and blood smear demonstrates a small lymphocyte with irregular nucleus (f).

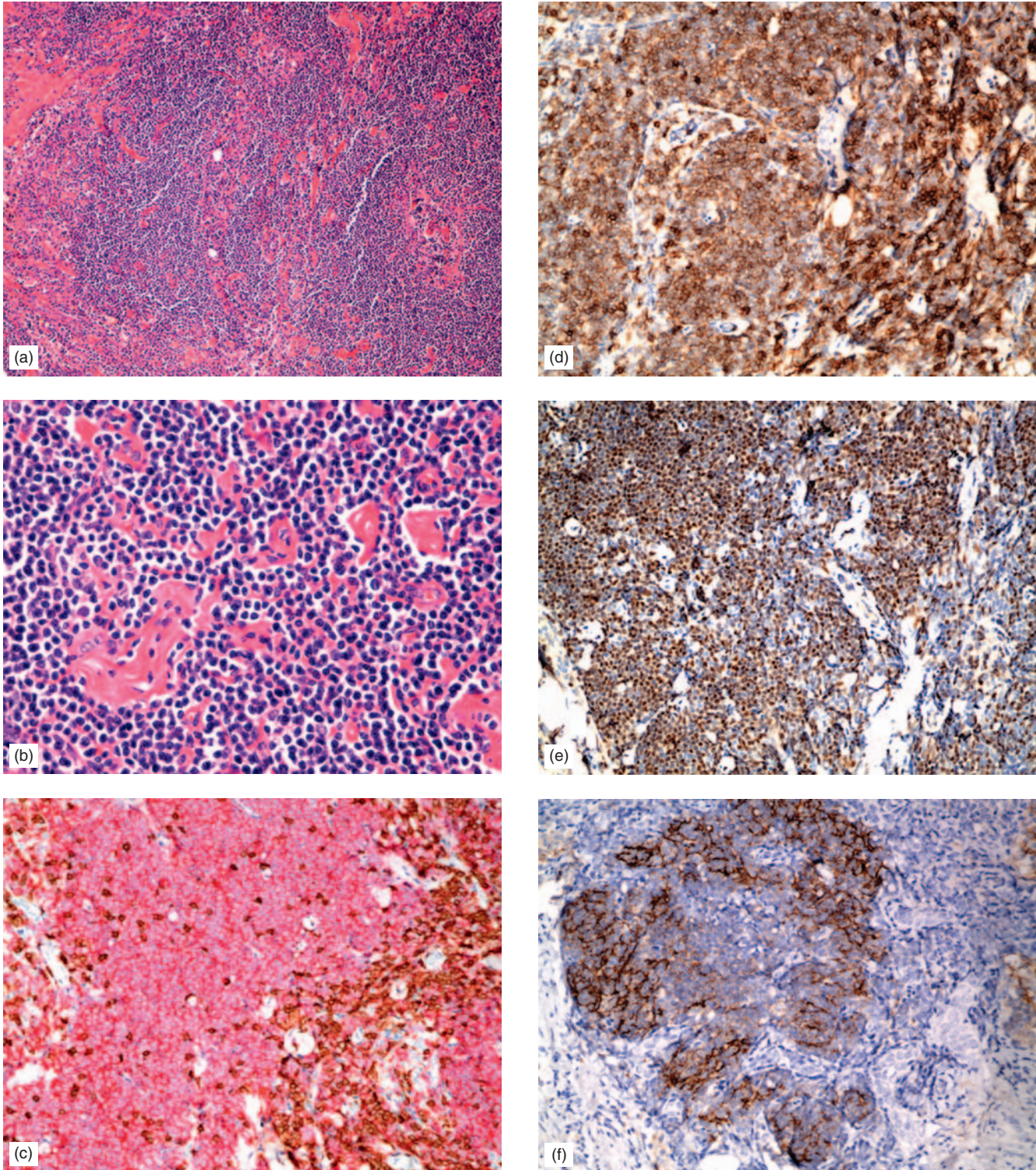


FIGURE 15.54 Mantle cell lymphoma. A lymph node section demonstrates sheets of small lymphocytes in a hyalinized fibrotic stroma: (a) low power and (b) high power views. Immunohistochemical stains show that these lymphocytes are predominantly B-cells (c: CD20+ = red, CD3+ = brown) and express CD5 (d) and BCL-1 (e). Aggregates of follicular dendritic cells are demonstrated expressing CD21 (f).

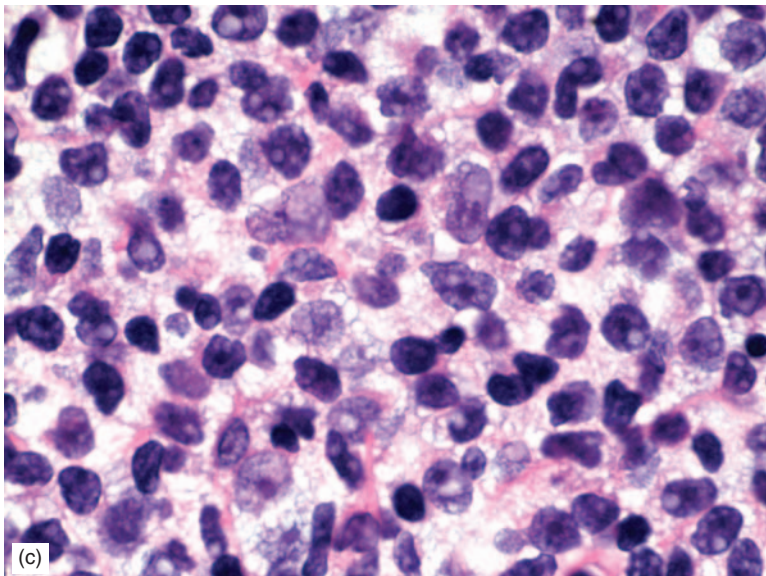
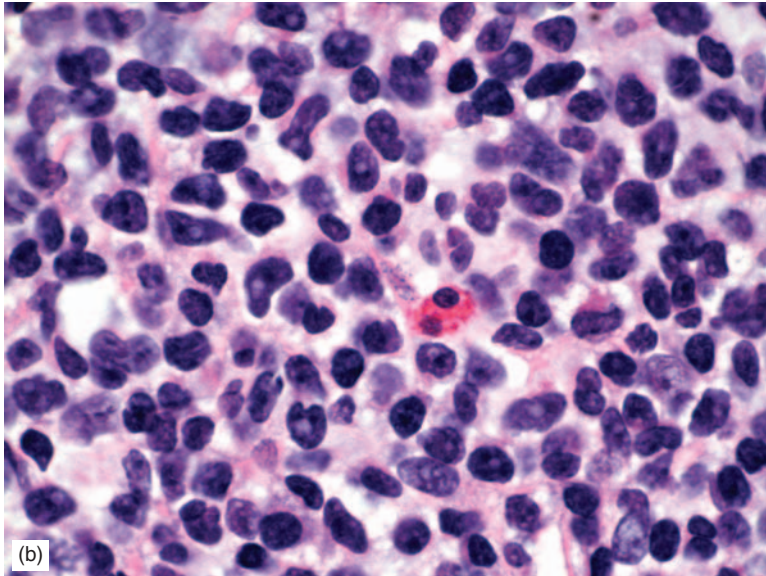
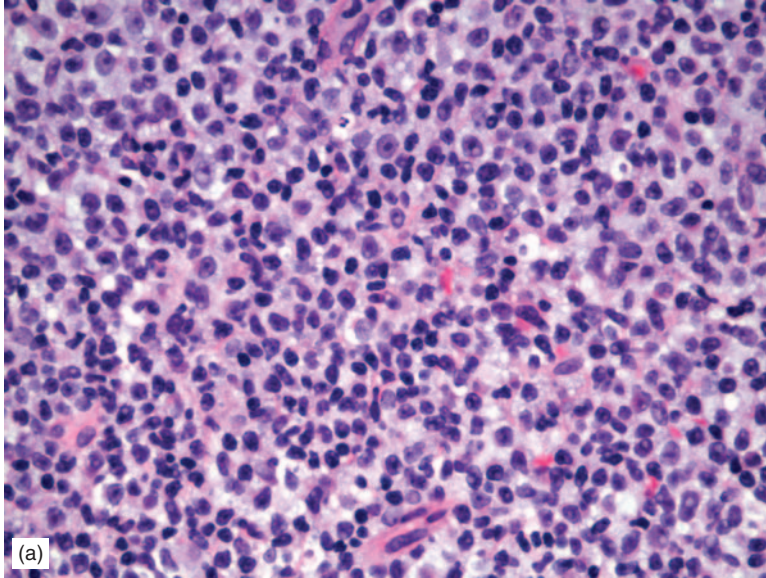


FIGURE 15.55 Blastoid variant of mantle cell lymphoma. Sheets of neoplastic cells demonstrating variable amounts of vacuolated cytoplasm, irregular nuclei with finely dispersed chromatin, and prominent nucleoli: (a) low power, (b) intermediate power, and (c) high power views.

TABLE 15.9 Cytologic variants of mantle cell lymphoma.*

Typical	Small to medium-sized lymphocytes with scant cytoplasm, slightly irregular nucleus, and condensed chromatin.
CLL-like	Small lymphocytes with scant cytoplasm, round nucleus, and condensed chromatin.
Monocytoid B-cell	Prominent foci of medium-sized cells with abundant pale cytoplasm.
Prolymphocyte-like	Medium-sized cells with variable amounts of cytoplasm, round or slightly irregular nucleus, relatively condensed chromatin and single prominent nucleus.
<i>Blastoid variants</i>	
Classic	Cells resembling lymphoblasts with high mitotic figure (>10/10hpf).
Pleomorphic	Pleomorphic large cells with variable amounts of cytoplasm, cleaved or oval nucleus. Nucleoli may be prominent.

*Adapted from Ref. [10].

except for the blastoid variant of MCL. The presence of a prominent meshwork of follicular dendritic cells is demonstrated by the expression of CD21 and/or CD35.

Cytogenetic and Molecular Studies

The characteristic cytogenetic alteration in MCL is the t(11;14)(q13;q32) (Figure 15.56). Classical cytogenetic studies detect t(11;14) in up to 65% of MCLs [220, 221]. But recent studies using FISH techniques have shown that this translocation is present in nearly all MCLs [222]. The t(11;14) is extremely rare in other lymphomas. It has been detected in occasional atypical cases of chronic lymphocytic leukemia (CLL) associated with an aggressive clinical course, as well as some cases of prolymphocytic leukemia, but in fact, these cases may represent atypical forms of MCL. Approximately 5% of patients with multiple myelomas show t(11;14) (see Chapter 16).

The main feature of MCL is overexpression of cyclin D1, which is due to the translocation of *BCL-1* to the heavy chain locus [223]. Cyclin D1 is a positive regulator of the G1/S cell-cycle restriction point and the overexpression induces increased cycling of the cells. Even if the overexpression of cyclin D1 is the main feature of MCL, it is probably not the sole oncogenic feature, as overexpression of the gene in mice fails to induce lymphomagenesis [224].

The breakpoints on 11q13 occur along a wide region with only 35% of these within a restricted region (major translocation cluster, abbreviated MTC). Thus, a PCR-based assay for the t(11;14) (*BCL1-IGH*) can detect the translocation only in a small fraction of MCL cases at genomic level (the translocation cannot be amplified at RNA level because there is no fusion product, only a deregulated expression of an otherwise normal *CCND1*), and PCR has a limited diagnostic role because of its high false-negative rate [225]. FISH is now considered the technique of choice for searching

for the t(11;14) during the diagnostic workup [226]. A new gene named *PRAD1* located approximately 120kb downstream of the MTC breakpoint was first identified in studies on parathyroid adenomas with inversion in chromosome 11, and this gene was considered to be a putative oncogene deregulated by t(11;14). In further studies, the *PRAD1* sequence was recognized as having a high degree of homology with cyclins, and the new member in that gene family was renamed *CCND1* encoding for the cyclin D1 protein [227]. In t(11;14), the coding region of *CCND1* is structurally intact, but the chromosomal rearrangement positioning the *CCND1* gene adjacent to the enhancer region of the immunoglobulin heavy chain gene results in upregulation of *CCND1* and in increased expression of the cyclin D1 protein. In normal lymphoid cells, the RNA and protein levels of cyclin D1 are extremely low or absent [228]. Although the oncogenic mechanism of cyclin D1 is not completely understood, the constant expression of cyclin D1 has an important role in the pathogenesis of MCL because cyclin D1 promotes the progression of cells through the main commitment checkpoint in G1- to S-phase of the cell cycle [229].

Although C-MYC mRNA overexpression has been found in a subset of MCLs, no structural gene alterations of the *C-MYC* seem to be involved in the pathogenesis of MCL. Some studies have shown p53 mutations and p53 protein overexpression to occur also in the aggressive variants of MCL, where the effect of overexpressed cyclin D1 may be enhanced by loss of cyclin-CDK inhibition as a result of the p53 mutation [230]. Structural and numerical centrosome abnormalities have been described to take place at a much higher frequency in MCL in comparison with other lymphoma subtypes, and this might explain their often near-tetraploid karyotype, especially among blastoid variants [231].

Several studies using comparative genomic hybridization (CGH) and/or FISH to characterize alterations in the frequency of DNA copy number sequences in MCL have been published [232, 233]. These studies have confirmed a characteristic profile of chromosomal changes in MCL different from that in other lymphomas. For example, in addition to trisomy 12, deletions in 13q, 11q, 6q, and 17p are the most frequent aberrations in CLL, whereas studies by CGH showed gains of chromosomes X, 1q, 7, and 3, together with losses in chromosomes 6q, X, and 1p in DLBCL [234]. In contrast, in MCL the most frequent chromosomal imbalances are gains of chromosomes 3q (49–70%), 8q (22–30%), and 12q (20–30%) and the most frequent losses of chromosomes 1p (24–33%), 6q (27–37%), 9p (16–41%), 11q (22–31%), and 13q (41–69%). DNA amplifications and the total number of copy number changes have been reported to be higher in the blastoid variant than in the common variant of MCL. In line with findings on the gene level, loss of chromosome 17p has been found to correlate with the rate of p53 inactivation, gain of 12q with the frequency of *CDK4* amplification, and high level amplification of chromosome 10p12-13 with BMI-1 amplification. Interestingly, further studies on the deletion 11q by FISH showed deletion of 11q22-23 in as many as 49% of MCL samples [235]. This region is known to harbor a number of tumor-associated genes, including the ataxia telangiectasia mutated (*ATM*) gene. These commonly affected

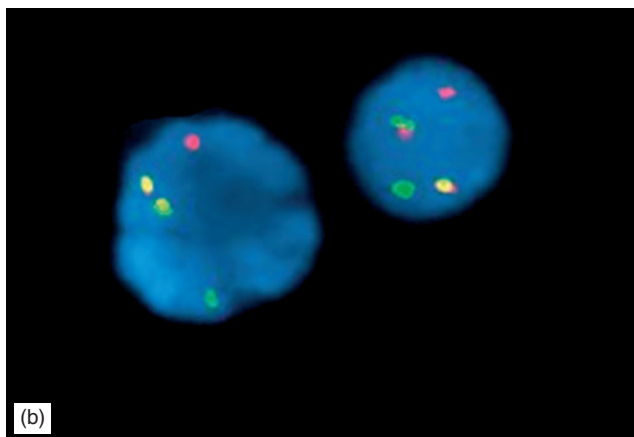
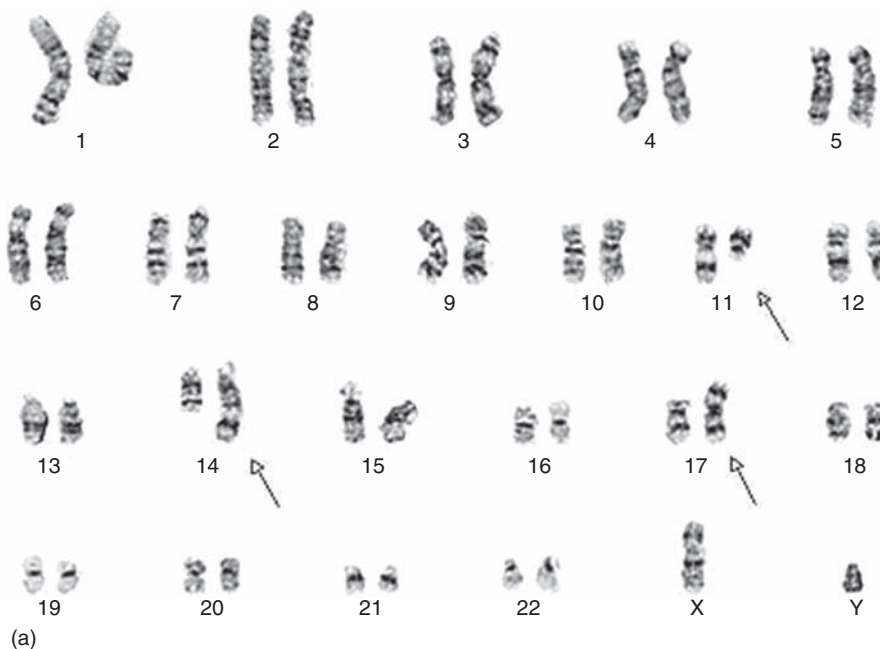


FIGURE 15.56 Karyotype of neoplastic cells from a patient with mantle cell lymphoma demonstrating 46, XY, t(11;14)(q13;q32), iso(17q) (a). FISH analysis shows cells with two fusion signals consistent with 11;14 translocation (b).

chromosomal regions may also harbor unknown oncogenes or tumor suppressor genes.

De Vos and colleagues found that several cell-cycle-related genes were deregulated when typical MCLs were compared with blastoid MCLs, such as *CDK4* gene, which was upregulated in the blastoid MCLs [236, 237]. *CDK4* cooperates with cyclin D1 in the progression through the G1/S checkpoint. Other cytogenetic abnormalities that were found when comparing typical and blastoid MCLs were deletions of *p53* and *p16*. Tumor cell proliferation has been shown to be associated with decreased survival time [238] and, as discussed earlier, is also predictive of blastoid transformation [239].

with a male:female ratio of about 3:1. Lymphadenopathy and splenomegaly (30–50%) are the major clinical findings and about 30% of the patients show systemic “B” symptoms [212–215]. MCL is considered an aggressive lymphoma with a median survival of 3–4 years. Patients with peripheral blood involvement, high mitotic figures, blastoid variants, and complex cytogenetic abnormalities show more aggressive clinical course [1, 240]. In one report, blastoid transformation occurred in 35% of patients with MCL with a median survival time of 4 months following transformation [241]. Therapeutic modalities such as combination chemotherapy and rituximab may improve the response rate, but without cure in most cases [242–245].

Clinical Aspects

MCL accounts for 5–10% of non-Hodgkin lymphomas in the United States and Europe. The median age is 63 years

Differential Diagnosis

The differential diagnosis includes CLL/SLL, FL, PLL, and marginal zone-related B-cell lymphomas. There are

overlapping cytologic features between CLL/SLL and MCL. Also, the vaguely nodular patterns in MCL and the pseudo-follicles in CLL/SLL may resemble one another. Unlike CLL, the neoplastic cells of MCL are monomorphic and lack prolymphocytes and paraimmunoblasts, usually lack the expression of CD23, express FMC7, CD79a, and BCL-1 nuclear protein, and demonstrate t(11;14).

The vague nodular pattern in MCL may mimic follicular pattern in FL. Unlike FL, the neoplastic cells of MCL lack centroblasts and immunoblasts, do not express CD10, are negative for t(14;18), express CD5, and demonstrate t(11;14).

MCL with monocytoid B-cell morphology may simulate marginal zone lymphomas of the spleen, lymph node, or extranodal sites. Marginal zone B-cells lack the expression of CD5, CD10, and CD23 and are negative for t(11;14).

The reported cases of PLL with t(11;14) are now considered *prolymphocytoid variant* of MCL [72, 246].

DIFFUSE LARGE B-CELL LYMPHOMA

Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin lymphoma consisting of large transformed neoplastic B-lymphocytes with prominent nucleoli, diffusely infiltrating the involved tissues [1, 247, 248].

Etiology and Pathogenesis

The etiology of DLBCL is not known. Immunodeficiency is a significant risk factor often in association with EBV infection. DLBCL is either *de novo* or the result of transformation of a less aggressive lymphoid malignancy [1]. DLBCL has also been reported in association with certain autoimmune disorders and chronic inflammatory conditions, such as Sjogren syndrome, rheumatoid arthritis and hepatitis C, and with HHV-8-positive multicentric Castleman disease [249].

The pathogenesis of this disorder appears to be complex with the involvement of a variety of genes including *BCL-6*, *PIM1*, *MYC*, *RoH/TTF (ARHH)*, and *PAX5*. *BCL-6* located at the 3q27 breakpoint may contribute to the lymphomagenesis by mediating the germinal center B-cell phenotype through transcriptional repression of the DNA-damage sensor ATR [250, 251]. The recent genetic profiling studies suggest two major groups in DLBCL: germinal center B-cell type and activated B-cell type. These two groups show different genetic profiles and chromosomal aberrations, and therefore probably different pathogenic pathways [252].

Pathology

Morphology

The neoplastic lymphoid cells are typically large cells (larger than a macrophage) and in most instances resemble centroblasts or immunoblasts or a mixture of the two [1, 2]. The involved lymph nodes show partial or total effacement of the nodal architecture with diffuse infiltration of large atypical neoplastic cells (Figure 15.57). Bone marrow

involvement is interstitial, diffuse, or nodular (Figure 15.58). The neoplastic cells may mimic clusters of megaloblasts or metastatic carcinoma. Peripheral blood smears may show the presence of neoplastic large cells. The most common extranodal primary site is the gastrointestinal tract, but skin, CNS, bone, spleen, liver, and other organs may be a primary site. Several morphologic variants have been described including centroblastic, immunoblastic, anaplastic, and T-cell/histiocyte-rich types [1, 2, 253].

Centroblastic variant: This variant is the most frequent type and consists of medium- to large-sized cells with scant amphophilic/basophilic cytoplasm, round or oval nucleus, fine nuclear chromatin, and several nucleoli bound to nuclear membrane (Figure 15.57). Multilobated (>3 lobes) centroblasts may be present and sometimes may create a polymorphic appearance. Some cases may show a mixture of centroblasts and immunoblasts.

Immunoblastic variant: This variant comprises about 10% of DLBCL and represents diffuse lymphomas with >90% immunoblasts. Immunoblasts are defined as large cells with abundant basophilic cytoplasm, round or oval nucleus, fine chromatin, and a prominent central nucleolus (Figure 15.59) [1, 254, 255]. Plasmacytoid features may be present. This variant is commonly seen in immune-compromised patients.

Anaplastic variant: This variant consists of atypical, large cells with bizarre pleomorphic nuclei, some of which may resemble Reed–Sternberg cells (Figure 15.60) [1, 256, 257]. These cells may appear in large clusters, resembling metastatic carcinoma. Sinusoidal pattern has been observed. The anaplastic variant of DLBCL does not share the distinctive clinicopathologic features of the T/null-anaplastic large cell lymphoma.

T-cell/histiocyte-rich variant: This is referred to those cases with heavy background of T-cells and often histiocytes admixed with scattered neoplastic large lymphocytes (Figure 15.61) [1, 258–261]. The neoplastic B-cells account for <10% of the total cells and may resemble Reed–Sternberg cells or variants. Histiocytes may appear epithelioid.

Immunophenotype

The neoplastic cells of DLBCL are commonly positive for B-cell-associated molecules, such as CD19, CD20, CD22, and CD79a, and express surface Ig (mostly IgM) (Figure 15.62) [1, 261, 262]. The BCL-6 nuclear protein is detected in about 70% of the cases, and 25–50% of the cases express BCL-2 [263]. Expression of CD5 or CD10 has been reported in 10% and 25–50% of the cases, respectively. The DLBCL cells are negative for BCL-1 [264]. The germinal center B-cell type of DLBCL is often associated with the expression of CD10 and BCL-6, whereas the activated B-cell type is usually positive for MUM1 (multiple myeloma1) and BCL-2 [265]. The majority of the cases of anaplastic variant of DLBCL are CD30-positive. The Ki-67 expression is high (>40%). Some cases of immunoblastic variant of DLBCL, particularly the AIDS-related ones, express MUM1 [265]. Occasional cases may demonstrate expression of the plasma-cell-associated marker CD138.

Cytogenetic and Molecular Studies

Numerous chromosomal aberrations, point mutations, and deletions have been described in DLBCL suggesting a

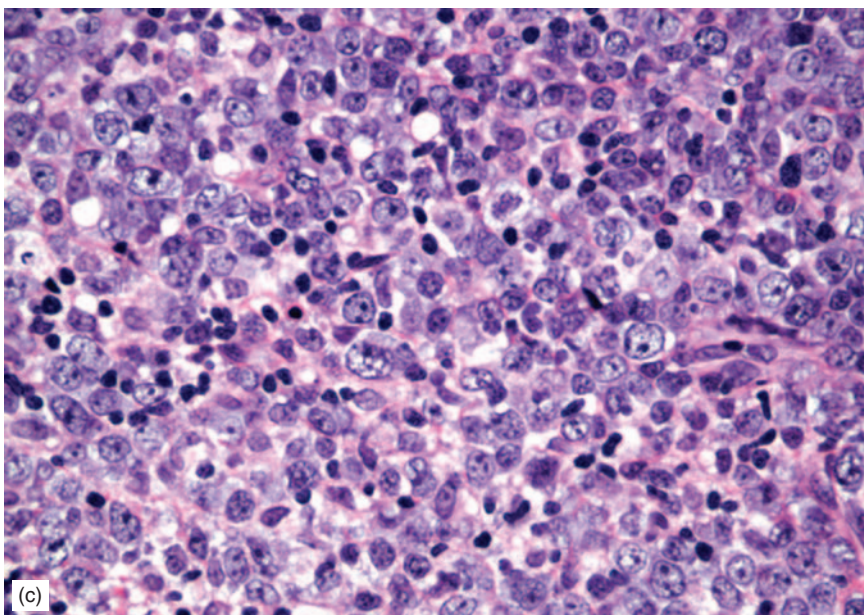
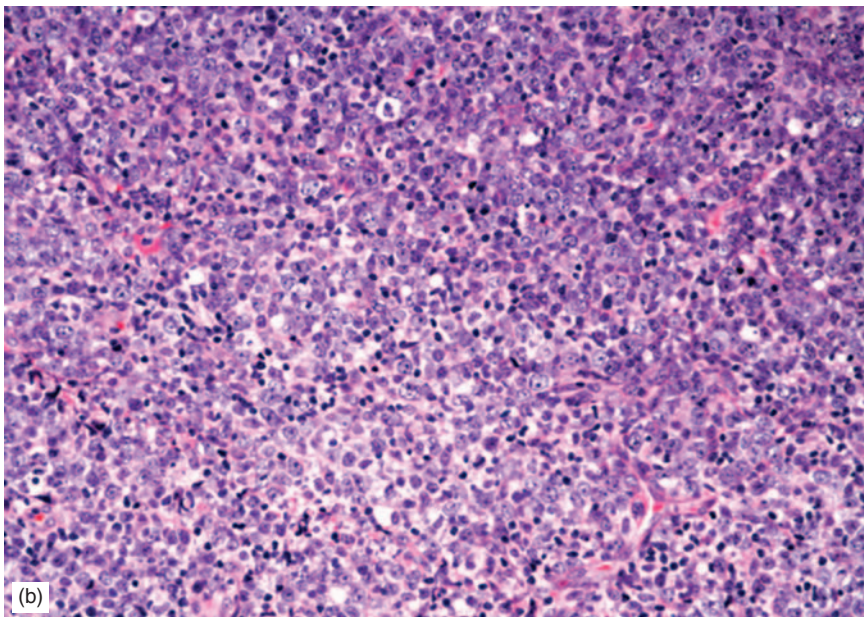
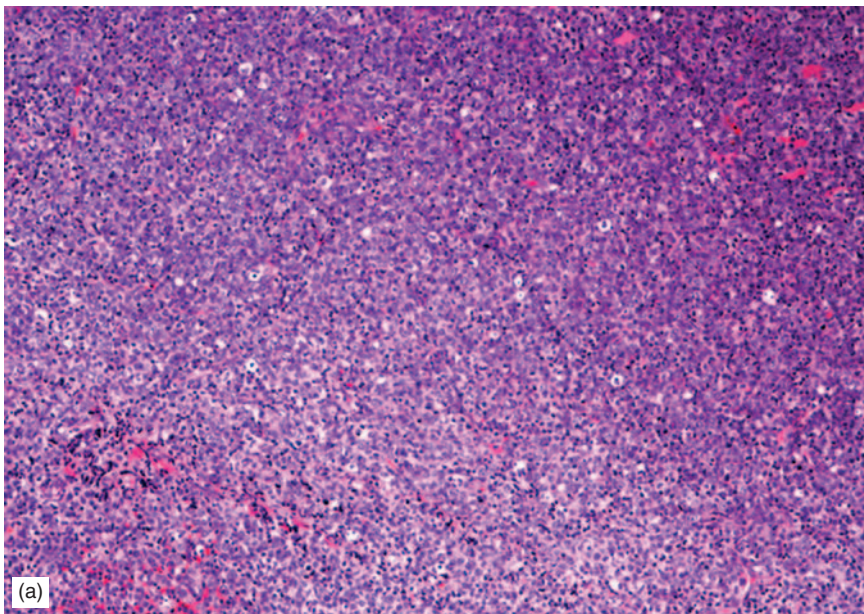


FIGURE 15.57 Lymph node section demonstrating diffuse large cell lymphoma: (a) low power, (b) intermediate power, and (c) high power views.

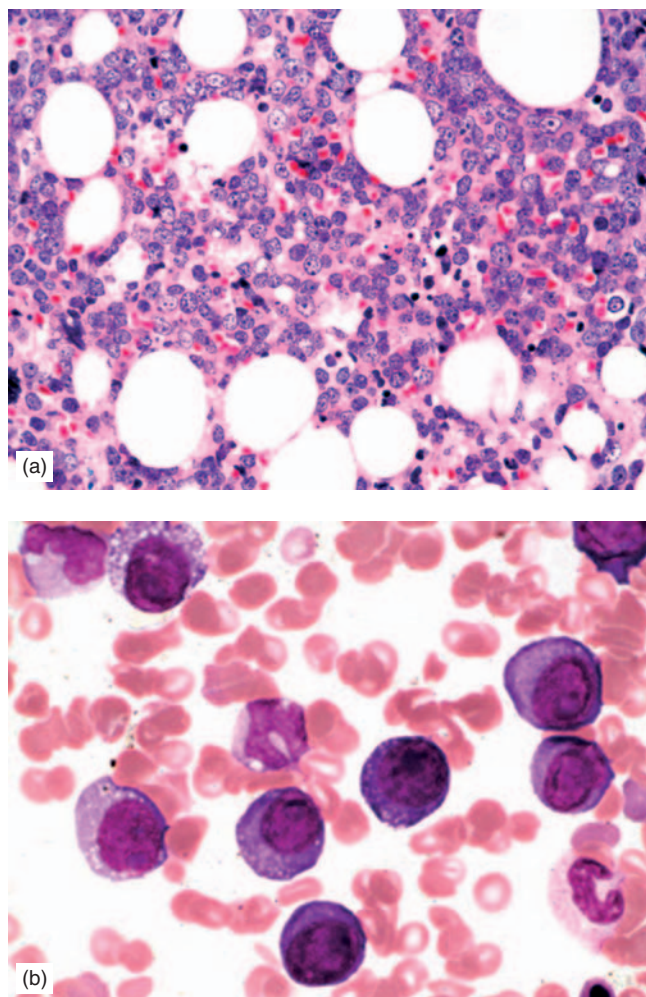


FIGURE 15.58 Bone marrow (a) and blood (b) involvement in a patient with diffuse large B-cell lymphoma.

heterogenous genetic background. The chromosomal translocation $t(14;18)(q32;q21.3)$ involving *BCL-2* is cytogenetically found in 20–30% of the cases [266]. However, overexpression of the *BCL-2* protein is found in 25–50% of the cases, suggesting that other mechanisms for the *BCL-2* overexpression are also involved [267]. Translocations involving the locus for *BCL-6* at 3q27 are found in almost a third of the cases [268]. Furthermore, mutations in the 5' regulatory region of the *BCL-6* gene are also seen, implying that *BCL-6* may be involved in the majority of DLBCL [269]. Clonal karyotypic abnormalities have been reported in up to 87% of the cases of DLBCL. The most commonly involved breakpoints are 14q32, 3q27, and 18q21, which are sites for *IGH*, *BCL-6*, and *BCL-2*, respectively. Other frequent chromosomal abnormalities include 1q36, 8q24, 3p21, 1p22, and 6q21 chromosomal bands. Complex cytogenetic abnormalities are frequent. *c-MYC* rearrangement (8q24) is rare and usually observed in immunodeficient patients. Mutations of *p53* and homozygous deletions at 9p21 are reported in lymphoma cases with large cell transformation [270].

Translocations affecting the immunoglobulin gene sites at 14q32 (*IGH*), 22q11.2 (*IGL*), and 2p12 (*IGK*) have

been identified in approximately half of DLBCL cases. These include $t(14;18)(q32;q21)$, $t(8;14)(q24;q32)$ or $t(8;22)(q24;q11.2)$, $t(3;14)(q27;q32)$, $t(3;22)(q27;q11.2)$, and other rearrangements involving 14q32. By conventional cytogenetics, FISH and CGH, the most common abnormalities observed in DLBCL are gains of chromosomes 2p (*REL*), 8q24 (*c-MYC*), chromosome 12 and 18q, losses of chromosomes 6q and 17p, and rearrangements of 3q27 (*BCL-6*) and 14q32 (*IGH*).

Genomic profiling studies demonstrate two major subgroups: one group shows characteristic features of germinal center B-cells (germinal center B-cell-like, or GCB-like) and the other group displays a profile similar to the activated B-cells (activated B-cell-like, or ABC-like) [252]. Gene profiling studies by Bea and associates in three groups of patients, DLBCL of GCB-like, DLBCL of ABC-like, and mediastinal large B-cell lymphoma, demonstrated significant differences in the frequency of particular chromosomal aberrations. For example, the group of DLBCL–GCB displayed frequent gains of chromosomes 12q, whereas the group of DLBCL–ABC had frequent trisomy 3, gains of chromosomes 3q and 18q21–q22, and losses of chromosome 6q21–q22. Other molecular profiling studies of DLBCL have revealed distinct subgroups with independent predictors of prognosis [271].

Clinical Aspects

DLBCL is the most common histological type and accounts for 30–40% of all non-Hodgkin lymphomas [2, 247, 248, 272]. The median age is about 64 years. The male:female ratio is slightly >1 . Most patients present with a rapidly enlarging mass, usually in the neck or abdomen. Extranodal involvement is observed in up to 40% of the patients, involving gastrointestinal tract, skin, CNS, bone, testis, liver, spleen, lung, and other organs. Approximately 20% of the patients are at stage I and about 40% show disseminated disease at the time of diagnosis. Bone marrow involvement is seen in 10–20% of the cases. The serum LDH levels are elevated in about 50% of the cases and approximately 30% of the patients show systemic “B” symptoms. A significant proportion of DLBCLs are the result of transformation of less aggressive lymphomas, such as CLL/SLL, LPL, SMZL, MALT lymphoma, and FL.

This lymphoma is considered an aggressive lymphoma. The IPI is highly predictive of the patients' clinical outcome. The clinical parameters used in the IPI include age >60 , elevated serum LDH, ECOG performance status ≥ 2 , stage III or IV, and number of involved extranodal site >1 . The 5-year survival rate is 26% and 73% for the high- and low-risk IPI groups, respectively [2].

Other adverse prognostic factors include the expression of CD5 and survivin protein [264, 273]. Survivin is the product of *BIRC5* gene and inhibitor of apoptosis. A gain in different regions of chromosome 3q has been associated with shorter survival [252]. On the contrary, expression of *BCL-6* nuclear protein has been reported to correlate with longer overall survival [274].

Therapeutic regimens include combination chemotherapy (e.g. CHOP) with or without involved field radiation for early stages and CHOP or other alternative

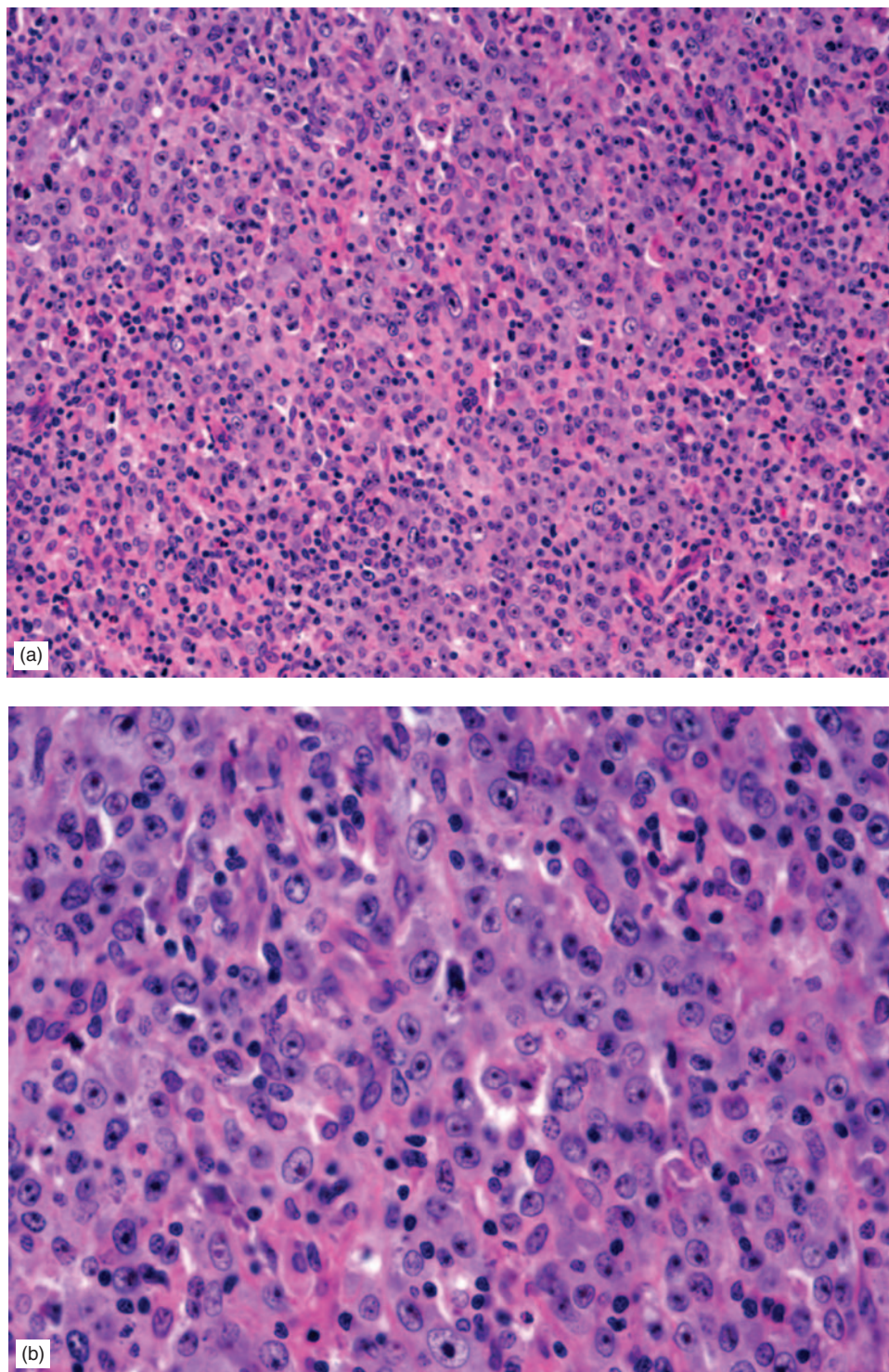


FIGURE 15.59 Lymph node section demonstrating diffuse large cell lymphoma, immunoblastic type: (a) low power and (b) high power views.

combination chemotherapy regimens with or without rituximab, or autologous transplantation in advanced or recurrent disease [2, 275, 276].

Differential Diagnosis

The centroblastic variant of DLBCL may show overlapping morphologic features with Burkitt-like lymphoma.

In general, the expression of Ki-67, CD10, and p53 is higher, and BCL-2 and adhesion molecules are lower in the neoplastic cells of Burkitt-like lymphoma than the DLBCL cells (Table 15.10) [277].

The extranodal immunoblastic and plasmablastic variants of DLBCL may mimic plasma cell myeloma. The differential diagnosis of T-cell/histiocyte-rich DLBCL includes lymphocyte predominance and mixed

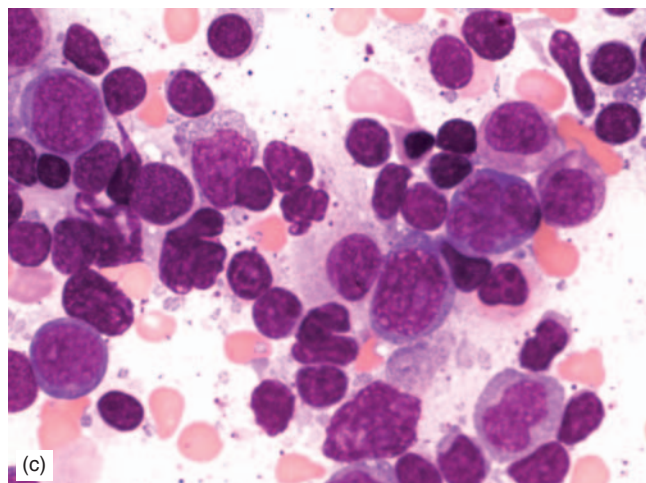
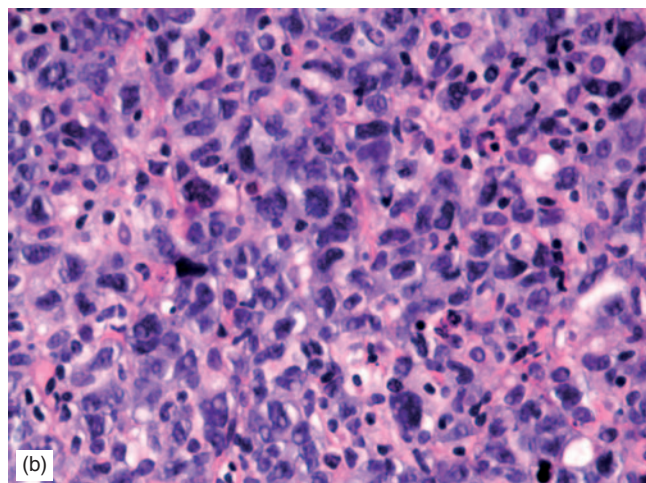
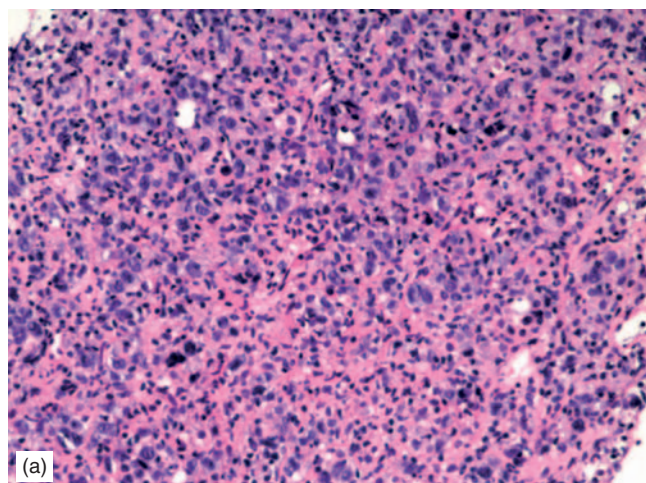


FIGURE 15.60 Bone marrow lymphomatous involvement in a patient with anaplastic large B-cell lymphoma: (a) low power and (b) high power views of biopsy section. Anaplastic cells are demonstrated in bone marrow smear (c).

cellularity Hodgkin lymphomas. The anaplastic variant of DLBCL shares morphologic features with T/null-anaplastic large cell lymphoma and classical Hodgkin lymphomas.

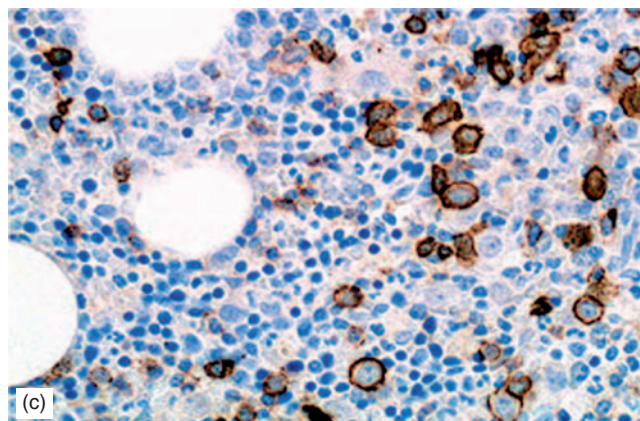
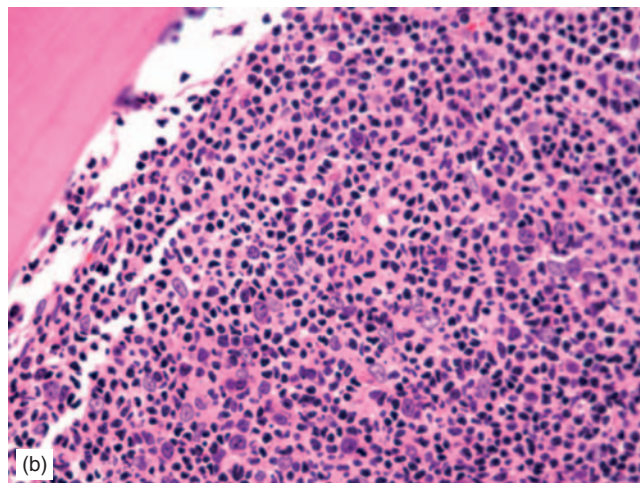
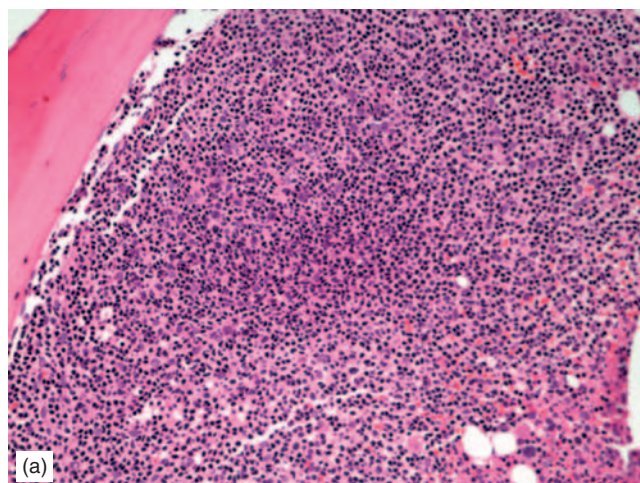


FIGURE 15.61 T-cell rich large B-cell lymphoma. Bone marrow biopsy section demonstrating scattered large cells mixed with a large number of small lymphocytes: (a) low power and (b) high power views. Large cells are CD20-positive and small cells are CD20-negative (c).

MEDIASTINAL LARGE B-CELL LYMPHOMA

Mediastinal (thymic) large B-cell lymphoma (MLBCL) is a distinct clinicopathologic subtype of DLBCL primarily involving the thymus [1, 278–280].

TABLE 15.10 Phenotypic characteristics of diffuse large B-cell lymphoma (DLBCL) and Burkitt-like lymphoma (BLL).*

Expression	DLBCL (%)	BLL (%)
CD19, CD20, and CD22	100	100
CD10	27	85
Mean Ki-67 fraction	53	88
p53	16	54
BCL-2	53	15
CD11a	87	38
CD18	85	10
CD44	86	8

*Adapted from Ref. [277].

Etiology and Pathogenesis

The etiology and pathogenesis of MLBCL are not known. Rare cases are associated with human herpesvirus 6 (HHV-6), raising the possibility that this virus may play a pathogenic role in a minority of the cases [278].

Pathology

Morphology

The neoplasm consists of large cells with variable amounts of cytoplasm, which is often pale or clear (Figure 15.63). The nuclear features may resemble centroblasts or large centrocytes, often with the presence of large cells with multilobated nuclei. Immunoblast-like cells may be dominant in minority of the cases. Reed–Sternberg-like cells may be present, as well as scattered eosinophils. Mitotic figures are frequent. Sclerosis is a common feature separating solid nests of tumor cells by thick hyalinized bands of connective tissue [2, 280–282].

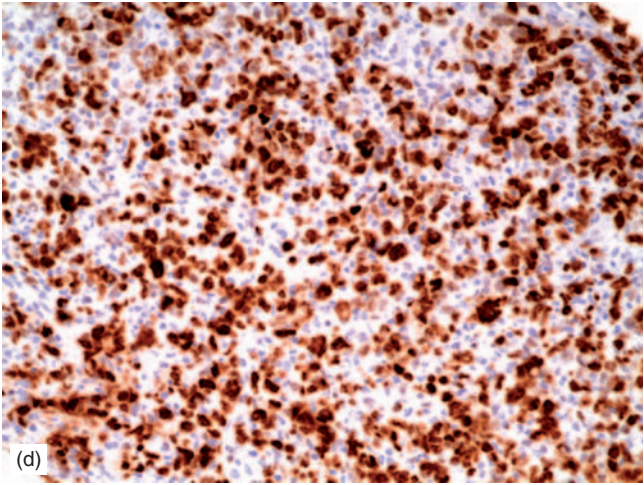
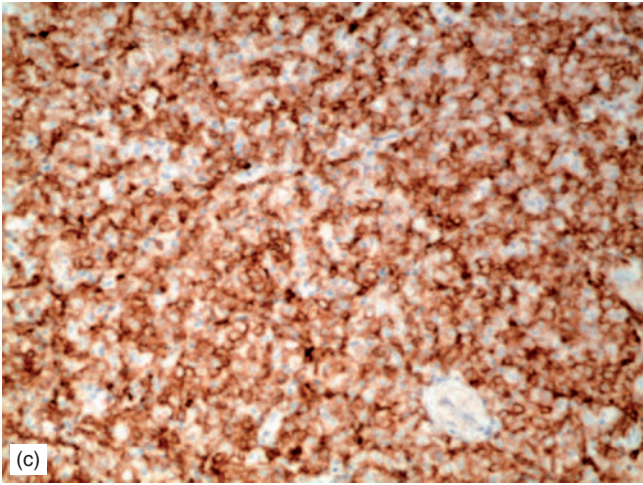
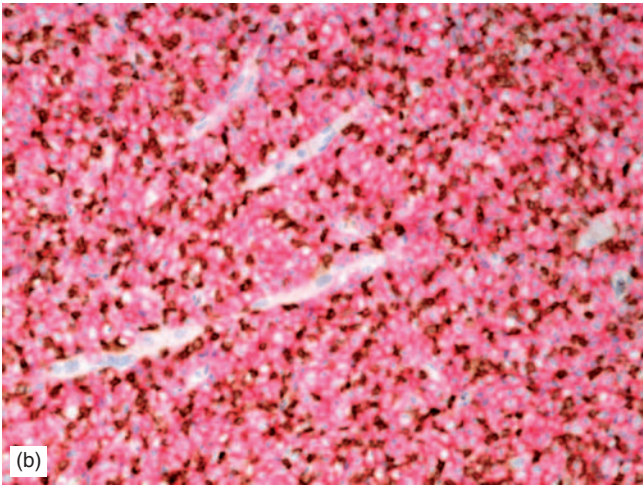
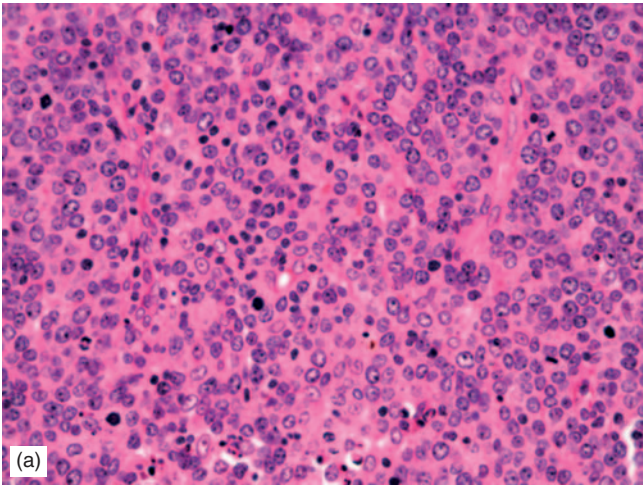


FIGURE 15.62 Immunophenotype of diffuse large B-cell lymphoma. The neoplastic cells show round nuclei with open chromatin and prominent nucleoli (a) and express CD20 (b, red), CD79a (c), and a high percentage of Ki-67 (d).

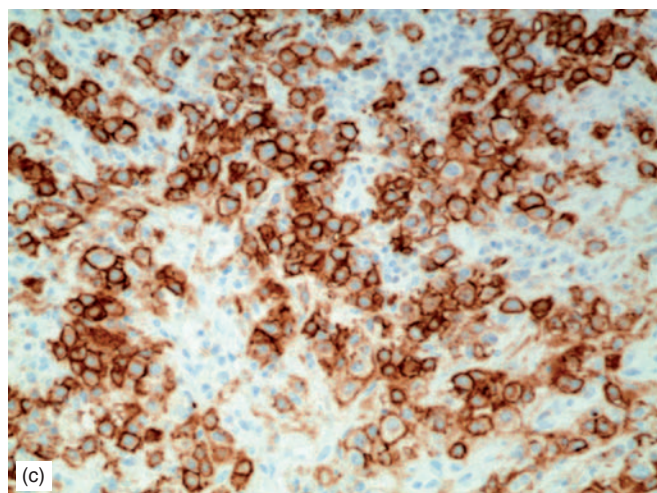
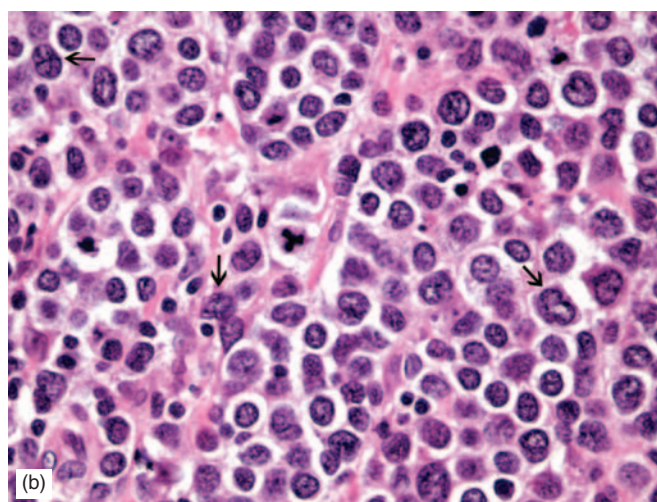
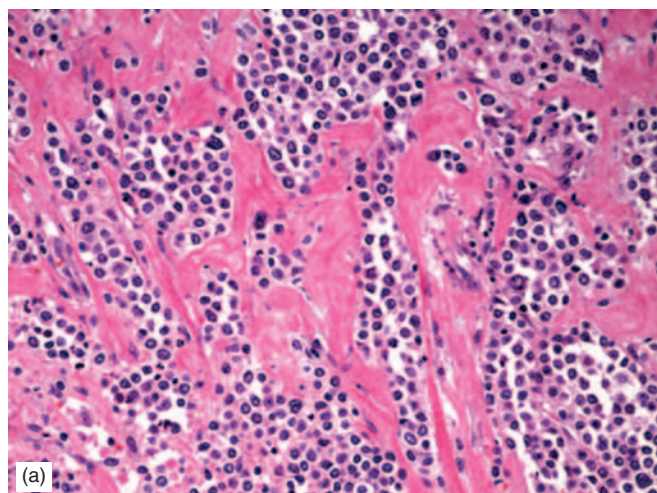


FIGURE 15.63 Primary mediastinal B-cell lymphoma. Aggregate of large neoplastic large cells are separated by thick bands of hyalinized connective tissue: (a) low power and (b) high power views. The neoplastic cells express CD20 by immunohistochemical technique (c).

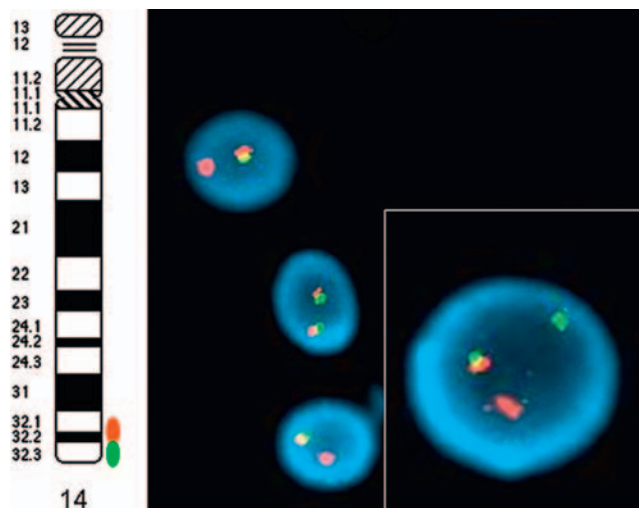


FIGURE 15.64 FISH analysis of neoplastic cells in a patient with primary mediastinal large B-cell lymphoma demonstrating *IGH* gene rearrangement (split red-green signals) (inset). Two of three other cells show 5'IGVH (green signal) deletion.

Immunophenotype

The neoplastic cells express B-cell-associated markers, such as CD19, CD20, CD22, and CD79a, and they may also express CD23 and CD30 [1, 2, 283, 284]. CD5 may be positive in minority of the cases and CD10 is negative. The multilobulated and Reed–Sternberg-like cells, unlike classic Reed–Sternberg cells, are negative for CD15 but express CD45.

Cytogenetic and Molecular Studies

The most frequent chromosomal abnormalities in MLBCL are gains of chromosomes 9p21 and 2p14-p16 [252]. Immunoglobulin gene rearrangement studies are usually positive, but *BCL-2*, *BCL-6*, and *MYC* genes lack rearrangements (Figure 15.64). *MAL*, a gene that encodes a protein associated with lipid rafts in the T-cells and epithelial cells, is overexpressed in majority of the MLBCL cases [283]. The *REL* gene, located at chromosomal position 2p16, is also frequently amplified [285].

Clinical Aspects

Primary MLBCL comprises about 7% of DLBCLs and 2.4% of all non-Hodgkin lymphomas [2]. Women are more affected than men, and the median age is around 40 years. The affected patients usually show a bulky anterior mediastinal mass originating in thymus. Superior vena cava syndrome is reported in over 50% of the cases. Relapses are often extranodal involving liver, gastrointestinal tract, CNS, and other organs. An event-free 10-year survival is about 50% [286]. Therapeutic regimens include CHOP or other alternative combination chemotherapy regimens followed by either involved field or modified mantle field radiation therapy.

Differential Diagnosis

The differential diagnosis of MLBCL includes nodular sclerosis Hodgkin lymphoma, thymoma, and other mediastinal

masses, such as seminoma and melanoma. The neoplastic cells of MLBCL express B-cell-associated markers and CD45, and lack the expression of CD15, cytokeratin, PLAP, and HMB-45.

OTHER VARIANTS OF LARGE B-CELL LYMPHOMA

Intravascular Large B-Cell Lymphoma

Intravascular large B-cell lymphoma (IVLBCL) is a rare and aggressive variant of extranodal large B-cell lymphoma

characterized by the presence of aggregates of large neoplastic B-cells within the lumina of small to medium-sized blood vessels [287–289]. The lack of expression of adhesion molecules, such as CD29 (beta 1 integrin) and CD54 (ICAM-1), in some cases of IVLBCL suggests that the intravascular pattern is secondary to a defect in homing receptors in the tumor cells [1, 290, 291].

Morphology

The neoplastic cells are large with large vesicular nucleus and prominent nucleoli. They appear as intraluminal clusters in the small to medium-sized vessels of many organs (Figure 15.65) [287–289, 292]. The tumor clusters may be associated with fibrin thrombi.

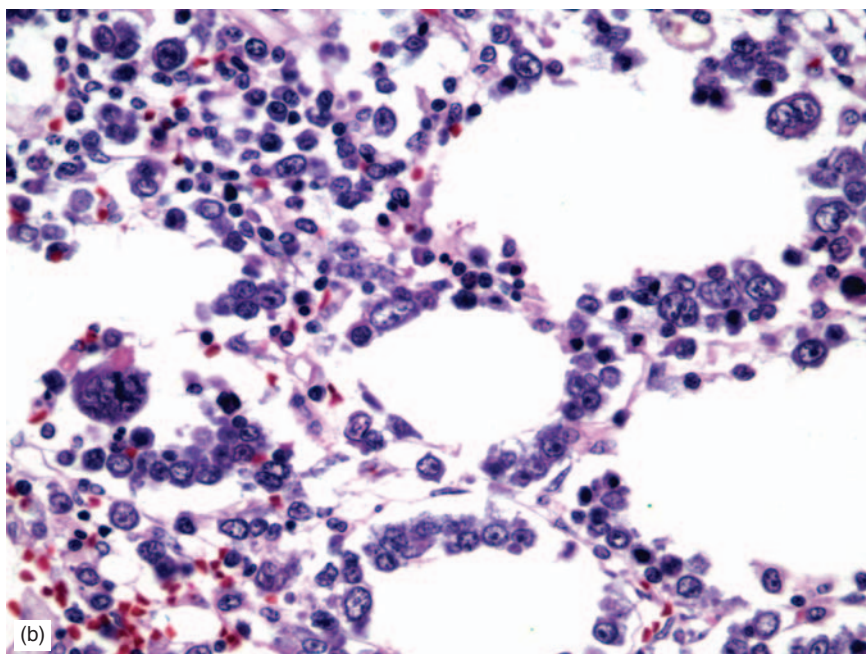
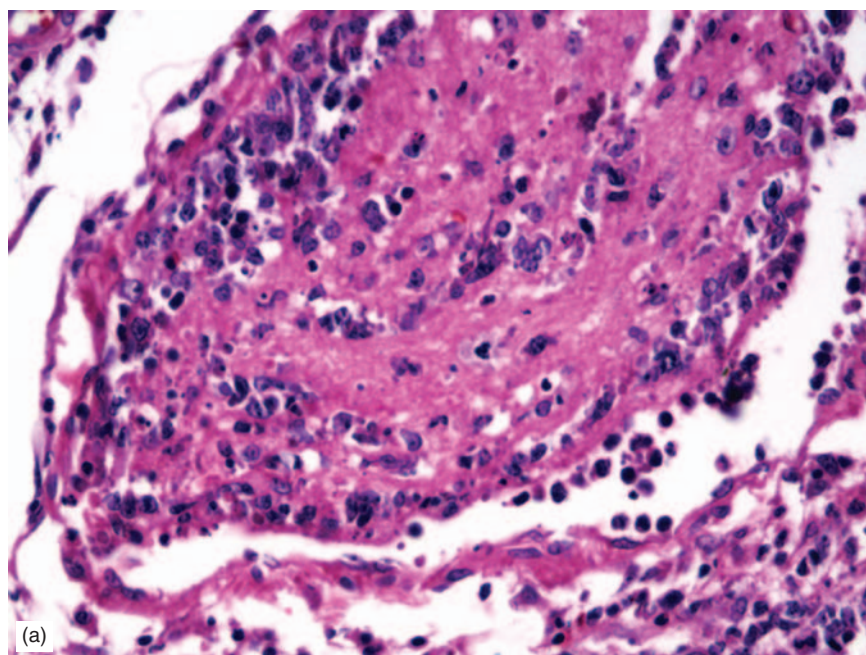


FIGURE 15.65 Intravascular accumulation of lymphoma cells (a) and their sinusoidal distribution in bone marrow (b).

TABLE 15.11 Immunophenotypic features of intravascular large B-cell lymphoma.*

Phenotype	% Positive
CD5	38
CD10	13
CD19	85
CD20	96
CD23	4
BCL-2	91
BCL-6	26
MUM1/IRF4	95
BCL-1	0
κ chain	71
λ chain	18
EBER (EBV)	0

*Adapted from Ref. [287].

Immunophenotype

The tumor cells are commonly positive for B-cell-associated molecules, such as Ig, CD19, CD20, CD22, and CD79a, and often express BCL-2 and MUM1 [287]. A smaller proportion of the cases may express CD5, CD10, and/or BCL-6. The BCL-1 expression is negative (Table 15.11).

Cytogenetic and Molecular Studies

No recurrent cytogenetic abnormalities have been reported so far. *BCL-6* rearrangement have been reported in sporadic cases [293]. Most cases show clonal *Ig* gene rearrangements.

Clinical Aspects

IVLBCL is an aggressive systemic disorder. Clinical symptoms are mostly secondary to the occlusion of the small to medium-sized vessels in various organs. Clinical manifestations may include skin lesions, neurological symptoms, nephritic syndrome, and disseminated intravascular coagulation (DIC). Hepatosplenomegaly, anemia, and thrombocytopenia are reported in over 70% of the patients [287]. In a recent report of 96 patients with IVLBCL, a correlation was found between CD5+, CD10− phenotype, and poor prognosis [287].

Primary Effusion Lymphoma

Primary effusion lymphoma is a rare variant of large cell lymphoma characterized by malignant serous effusions without detectable tumor masses [1, 294]. Rare cases may show solid organ involvements, such as skin and heart [295, 296]. It has been primarily observed in AIDS patients and

is associated with HHV-8 [294, 297–299]. However, rare cases of HHV-8-negative PEL have been reported [300, 301]. The majority of cases are also co-infected with EBV [1, 294]. Pleural, pericardial, and peritoneal cavities are the most frequent sites of involvement.

Morphology

The cytocentrifuge slide preparations of the lymphomatous effusions show large neoplastic cells with immunoblastic, plasmablastic, and/or anaplastic features (Figure 15.66) [1, 294, 302]. These cells have variable amounts of basophilic cytoplasm. A perinuclear pale area may be present (plasmablastic). Nuclei are round or irregular and show prominent nucleoli. Binucleated or multilobated nuclei may be present and some tumor cells may resemble Reed–Sternberg cells.

Immunophenotype

The tumor cells express CD45 but are usually negative for surface and cytoplasmic Ig and B-cell-associated markers, such as CD19, CD20, and CD79a [1, 302–304]. They often express CD30, CD38, and CD138, and in some cases may show aberrant expression of cytoplasmic CD3. Immunohistochemical stains are positive for HHV-8-associated latent protein.

Cytogenetic and Molecular Studies

No recurrent cytogenetic abnormalities have been reported. Although the tumor cells often lack expression of membrane or cytoplasmic Ig, *Ig* genes are rearranged and mutated, so molecular studies are more necessary here than in many of the other B-cell lymphomas [1, 303]. HHV-8 viral genomes are detected in virtually all patients, and most cases show EBV infection demonstrated by EBER [249, 305]. Some cases may also show *TCR* gene rearrangement. A case of PEL of the pericardial cavity carrying t(1;22)(q21;q11.2) and t(14;17)(q32;q23) has been reported [306].

Clinical Aspects

The majority of patients are men and have contracted HIV. Clinical symptoms are secondary to the effusions [303]. Some patients may demonstrate Kaposi sarcoma or multicentric Castleman disease [249]. Most patients do not respond to conventional chemotherapy and have a short survival time, usually <6 months.

Plasmablastic Lymphoma

Plasmablastic lymphoma is a rare type of LBCL reported in the HIV-infected patients [307–309]. In the majority of the cases, the neoplastic cells are positive for EBV and HHV-8. Plasmablastic lymphoma is often preceded by multicentric or plasmablastic variant of Castleman disease.

Morphology

The tumor cells are large and resemble immunoblasts or atypical immature plasma cells with variable amounts of amphophilic or basophilic cytoplasm, round or irregular

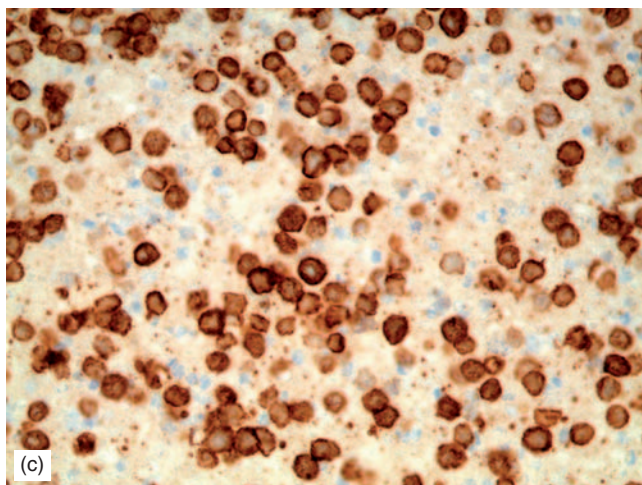
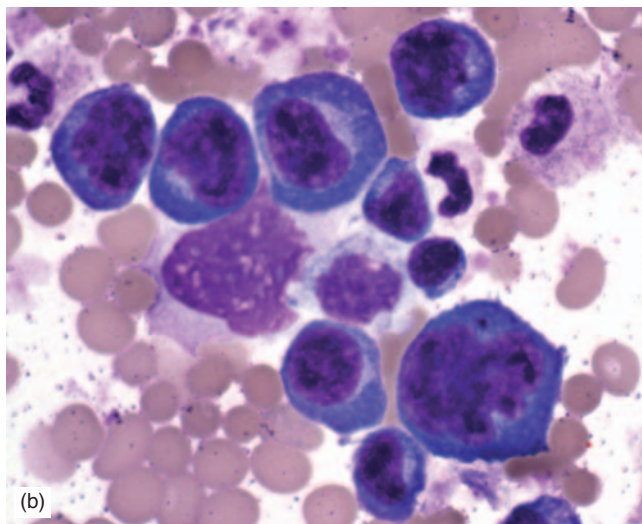
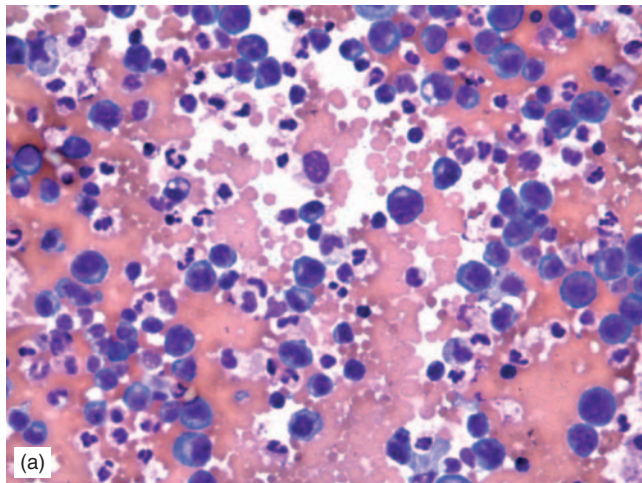


FIGURE 15.66 Primary effusion lymphoma. Pericardial effusion demonstrating large plasmablastic cells with dark-blue cytoplasm and irregular nuclei mixed with red cells and inflammatory cells: (a) low power and (b) high power views. These cells express CD138 by immunohistochemical stains (c).

vesicular nucleus and one or more prominent nucleoli (Figure 15.67) [1, 310].

Immunophenotype

These cells, similar to PEL cells, are usually negative for B-cell-associated markers, such as CD19, CD20, and CD79a, but positive for CD38 and CD138. They often lack CD45 expression but show a high Ki-67 fraction [1, 249]. Immunohistochemical stains are positive for HHV-8-associated latent protein (Figure 15.67).

Cytogenetic and Molecular Studies

No recurrent cytogenetic abnormalities have been reported. The *Ig* genes are clonally rearranged. HHV-8 viral genomes are detected in virtually all patients, and most cases show EBV infection demonstrated by EBER.

Clinical Aspects

This tumor typically involves the oral cavity of AIDS patients, though extra-oral cases have also been reported [307–309, 311]. The affected patients may demonstrate Kaposi sarcoma or multicentric Castleman disease [249].

Anaplastic Lymphoma Kinase Positive DLBCL

Anaplastic lymphoma kinase (ALK)-positive DLBCL is a rare, recently defined lymphoma comprised of large anaplastic cells with immunoblastic and/or plasmablastic morphologic features [256, 312, 313]. The tumor cells usually have an intrasinusoidal distribution and express cytoplasmic ALK, CD138, CD45, EMA, and monoclonal cytoplasmic light chain and weak IgA [256, 312, 313]. Other B-cell-associated markers are usually negative, as well as CD3, CD30, CD56, and TIA-1. Cytogenetic studies may reveal $t(2;5)(p23;q35);(ALK/NPM1)$ or $t(2;17)(p23;q23);(ALK/CLTC)$ [256, 312].

DLBCL Associated with Chronic Inflammation

Certain chronic inflammations such as autoimmune disorders, chronic pyothorax, and hepatitis C virus are sometimes associated with DLBCL [314–316]. The neoplastic cells in these cases express CD20 and TIA-1 and demonstrate clonal *IGH* and/or *TCR* gene rearrangements.

Primary Central Nervous System DLBCL

Primary central nervous system (CNS) lymphomas are aggressive tumors confined to the CNS [317–319]. These lymphomas are usually of large B-cell type and are distinguished from nodal DLBCL by high expression of regulators of the protein response (UPR), and interleukin-4 (IL-4) [319]. More recent studies show upregulation of the ECM (extracellular matrix)-related osteopontin gene, *SPP1* in primary CNS large B-cell lymphomas [317].

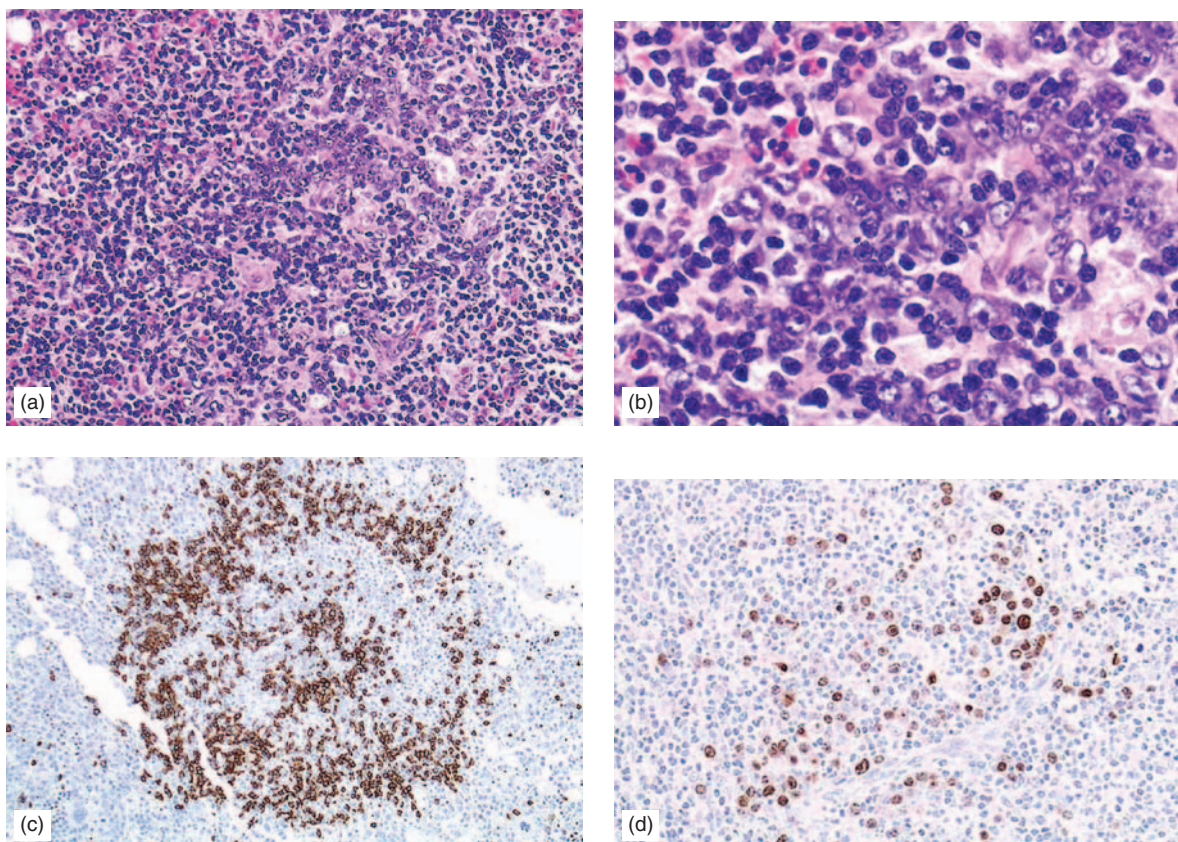


FIGURE 15.67 Plasmablastic lymphoma in a patient with AIDS. Aggregates of immature cells (plasmablasts) are present: (a) low power and (b) high power views. These cells express CD20 (c) and are positive for HHV-8 (d).

Primary Cutaneous DLBCL

Primary cutaneous DLBCL, leg type, is one of the categories in cutaneous lymphomas defined by the World Health Organization–European Organization for Research and Treatment of Cancer Classification [320–324]. These tumors in addition to the expression of B-cell-associated markers are often CD10+ and BCL-6+ and may demonstrate t(14;18) translocation by PCR [321].

BURKITT LYMPHOMA

Burkitt lymphoma/leukemia is a highly aggressive mature B-cell lymphoid malignancy consisting of endemic, sporadic, and immunodeficiency-associated variants [323–325]. All variants demonstrate chromosomal rearrangements involving *C-MYC* oncogene and share morphologic and immunophenotypic features, but differ in clinical and geographic presentations [326].

Etiology and Pathogenesis

The strong association of EBV with the endemic variant of BL suggests an important etiologic role for this virus.

Virtually, all patients with endemic BL show the EBV genome in their neoplasm. The assumption is that recurrent or chronic viral (EBV, HIV), bacterial, or parasitic infections (malaria) lead to the development of lymphoma due to defective T-cell regulation of EBV-infected B-cells [1, 327] and increased chance of *C-MYC* translocation. In the sporadic and immunodeficiency-associated BL, EBV infection is detected in <30% and 25–40% of the tumors, respectively [1].

C-MYC overexpression plays an important role in the pathogenesis of BL. Numerous genes are induced by overexpression of *C-MYC* including *cyclin D2*, *TRAP1* (apoptosis gene), *HLA-DRBI* histocompatibility gene, and the mitochondrial *HSPD1* (heat shock 60 kD protein). *C-MYC* overexpression also regulates a number of CDKs, such as CDK2 and CDK4, and other cell cycle-related products including *CCN D1*, *p27*, and *p53* [323, 328, 329]. In summary, the upregulation of *C-MYC* oncogene promotes cell cycle progression, inhibits the differentiation process, alters cellular metabolism, reduces cell adhesion, and increases telomerase activity (Figure 15.68).

Pathology

Morphology

The involved tissues are diffusely infiltrated by sheets of monotonous medium-sized neoplastic lymphoid cells. The

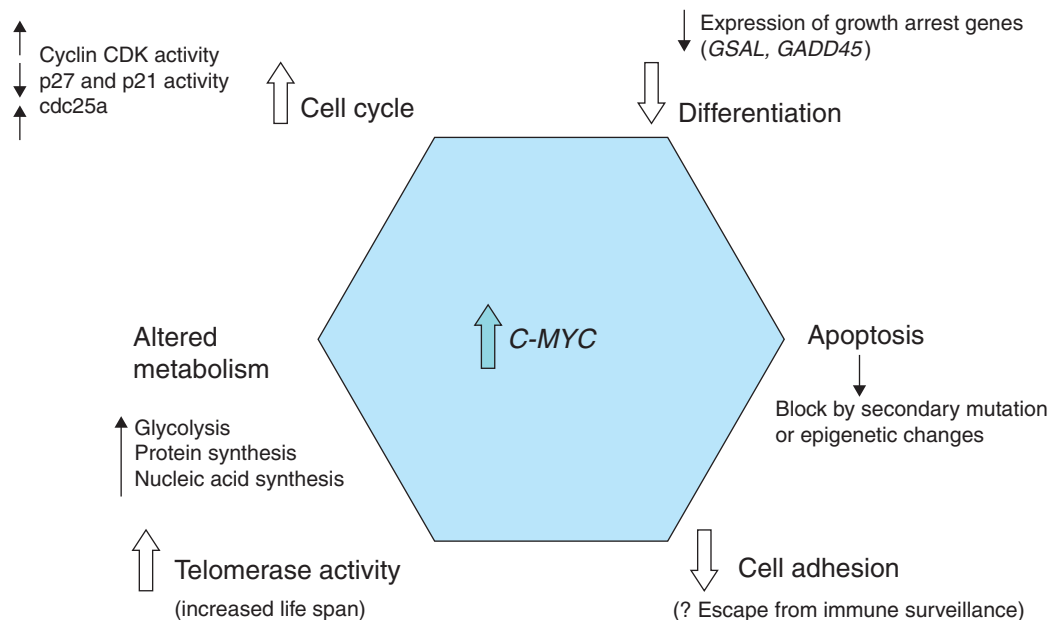


FIGURE 15.68 Effects of *C-MYC* overexpression in various aspects of cell function. Adapted from Hecht JL, Aster JC. (2000). Molecular biology of Burkitt's lymphoma. *J Clin Oncol* **18**, 3707–21.

typical (classical) cytologic features are uniformity, high nuclear:cytoplasmic ratio, basophilic and often vacuolated cytoplasm, round nucleus with clumped chromatin and relatively clear parachromatin, and multiple centrally located nucleoli [1, 2, 93, 324]. Mitotic figures are numerous as well as a high rate of apoptosis. Scattered macrophages with abundant, pale, cytoplasm-containing cell debris are present, creating a “starry sky” pattern (Figure 15.69). BL may infiltrate bone marrow and/or peripheral blood and present a leukemic picture (Figure 15.70).

The WHO has defined two morphologic variants [1]:

1. *Atypical Burkitt/Burkitt-like*: The neoplastic cells in atypical Burkitt or Burkitt-like variant are pleomorphic and show variation in the nuclear size and shape. The nucleoli are fewer in number, but more prominent [1, 277].
2. *BL with plasmacytoid differentiation*: Some neoplastic cells display plasmacytic differentiation with basophilic cytoplasm, eccentrically located nucleus, and a single central nucleolus. This variant is more common in patients with immunodeficiency.

Immunophenotype and Cytochemical Stains

Neoplastic cells of BL express membrane IgM, are membrane Ig light chain restricted, and are positive for B-cell-associated markers, such as CD19, CD20, CD22, and CD79a (Figures 15.71 and 15.72) [1, 2, 93, 330]. They express CD10, CD43, and BCL-6 and lack expression of CD5, CD23, and BCL-2. Virtually all the viable neoplastic cells express Ki-67. The EBV-positive cases (e.g. endemic type) express CD21. BL cells usually lack the expression of adhesion molecules, such as CD11a, CD11c, CD18, and CD44. CD34 and TdT are negative. The BL cells show positive staining for oil red O (Figure 15.73).

Cytogenetic and Molecular Studies

The genetic hallmark of BL is the translocation of *MYC* at chromosome 8q24 [1, 2, 93, 330]. This translocation observed in a majority of the cases is between *MYC* and the Ig heavy chain region on chromosome 14 [t(8;14)(q24;q32)] (Figure 15.74), but less frequently, it may involve Ig light chain regions on chromosomes 2 (kappa) and 22 (lambda), t(2;8)(p12;q24) and t(8;22)(q24;q11.1).

In the endemic cases, the breakpoint on chromosome 14 involves the joining region of Ig heavy chain, and the breakpoint on chromosome 8 lies outside the *c-MYC* gene, whereas in the sporadic BL, the breakpoints are in the heavy chain switch region and inside the *c-MYC* gene [2]. In fact, the heterogeneity of the molecular breakpoints makes this translocation difficult to detect by standard PCR approaches, and cytogenetics or FISH are preferred [331, 332]. Mutation of *BCL-6* gene has been reported in 25–50% of the BL cases. Most endemic cases and 25–40% of the cases associated with AIDS contain EBV genome [2].

Clinical Aspects

Three major clinical forms of BL have been described: endemic, sporadic, and immunodeficiency associated [2, 323–325]. The differences are primarily based on epidemiology and clinical presentations. The male:female ratio is about 2–4:1 in all three forms. The endemic and sporadic forms are most common in children, and affected children with the endemic form are younger than the patients with sporadic form.

Endemic BL occurs in equatorial Africa. It is the most common childhood malignancy in this region with a peak incidence at 4–7 years. There is some correlation between the incidence of endemic BL and geographical

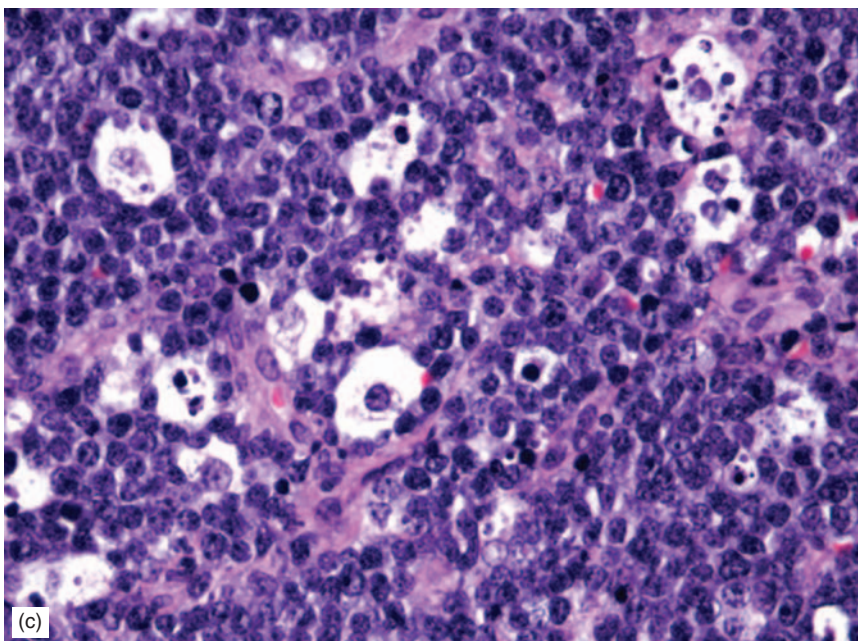
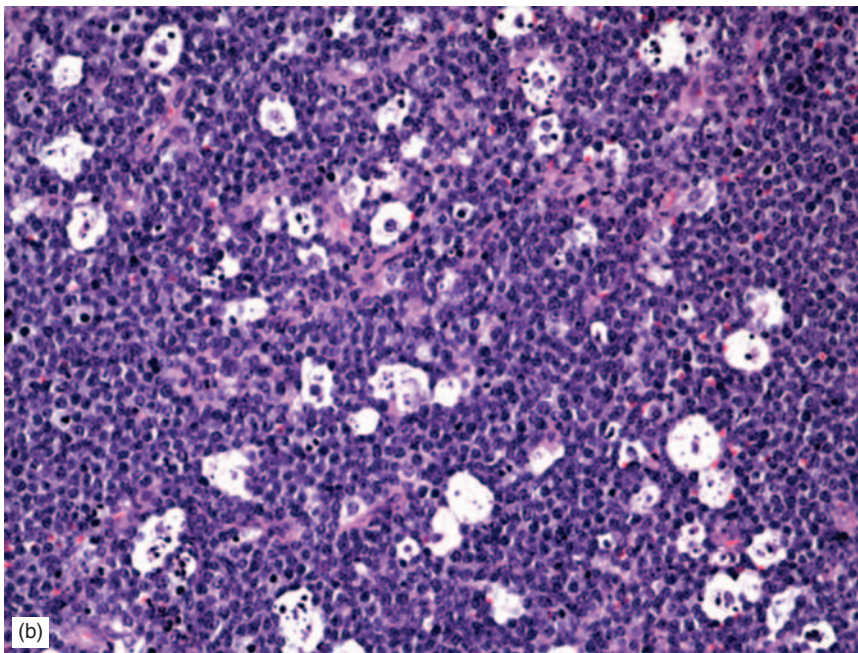
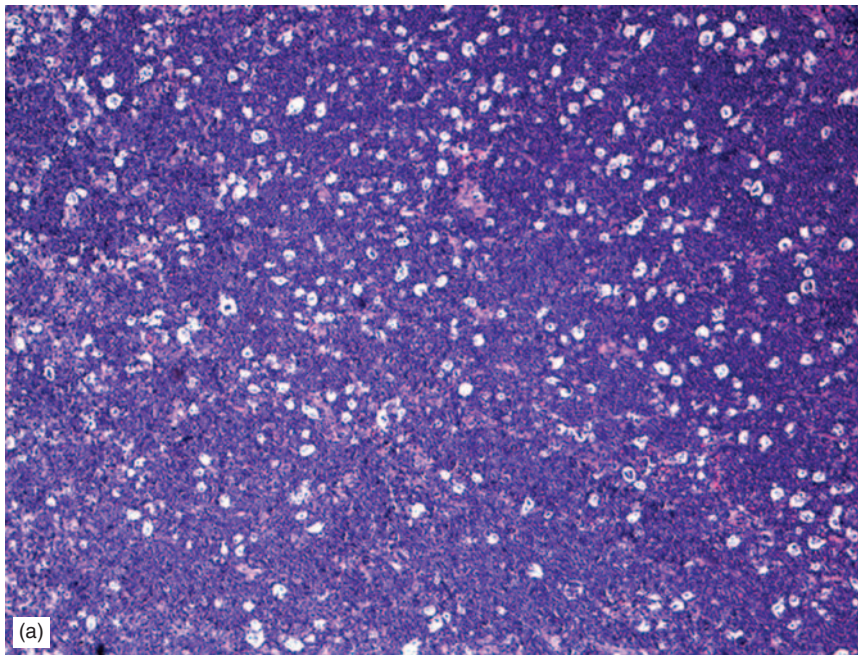


FIGURE 15.69 Burkitt lymphoma. A lymph node section demonstrating a diffuse infiltration by monomorphic lymphoid cells with high nuclear: cytoplasmic ratio, basophilic cytoplasm, round nucleus with clumped chromatin and relatively clear parachromatin, and multiple centrally located nucleoli. Scattered macrophages with abundant pale cytoplasm-containing cell debris are present, creating a "starry sky" pattern: (a) low power, (b) intermediate power, and (c) high power views.

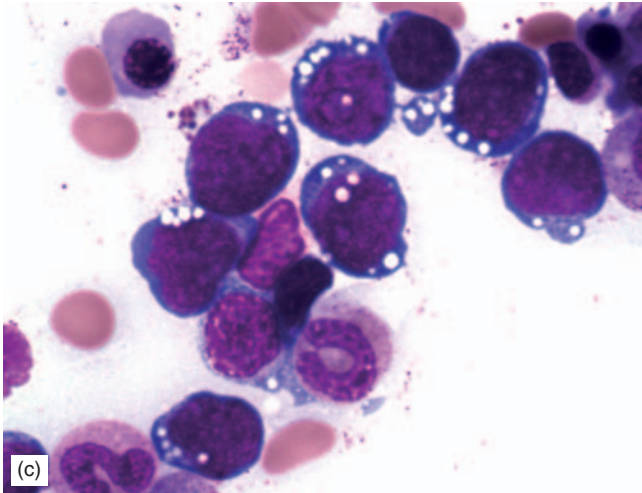
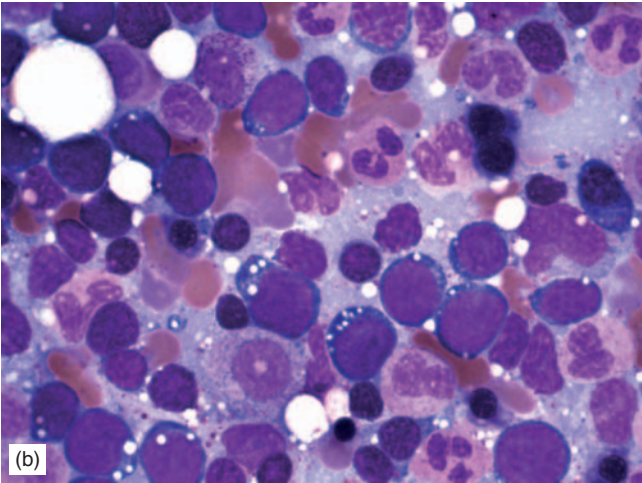
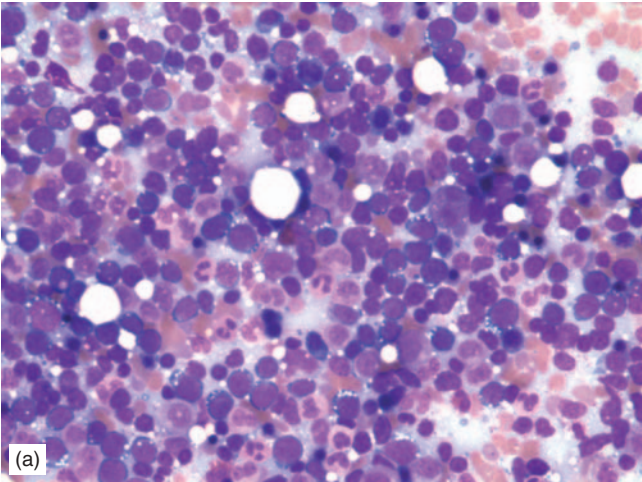


FIGURE 15.70 Burkitt lymphoma. Bone marrow smear showing monomorphic neoplastic lymphoid cells with high nuclear:cytoplasmic ratio, basophilic vacuolated cytoplasm, and round nuclei: (a) low power, (b) intermediate power, and (c) high power views.

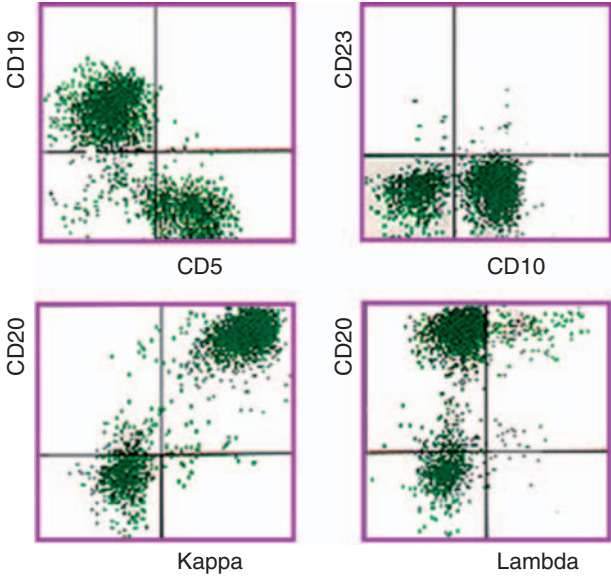


FIGURE 15.71 Flow cytometric analysis of neoplastic cells of Burkitt lymphoma demonstrates a population of B-cells expressing CD10, CD19, and CD20 with kappa light chain restriction.

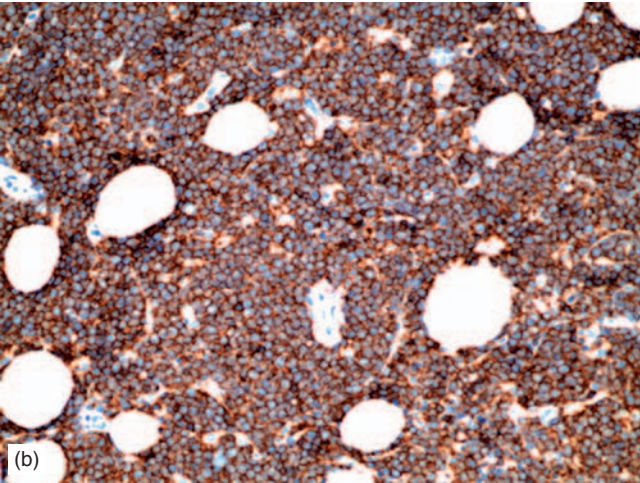
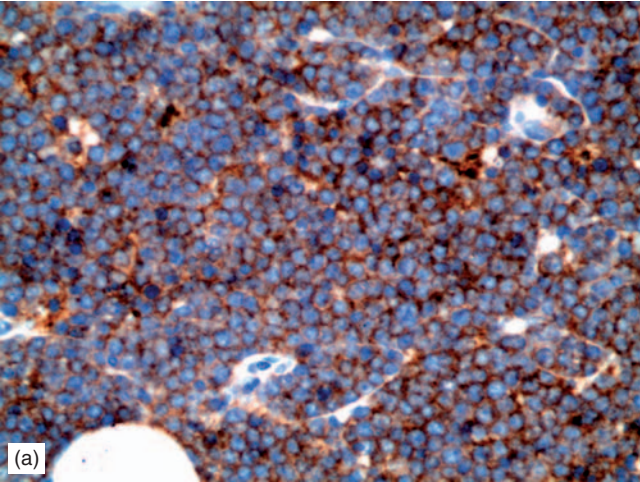


FIGURE 15.72 Burkitt lymphoma: immunohistochemical stains. The tumor cells express CD10 (a), CD20 (b), Ki-67 (c), and BCL-6 (d).

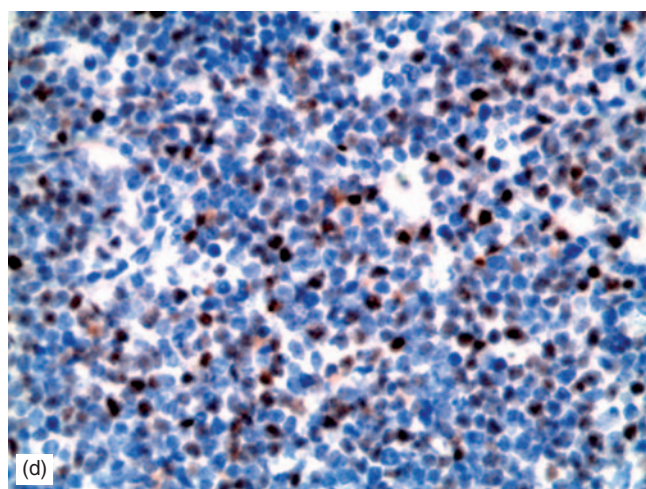
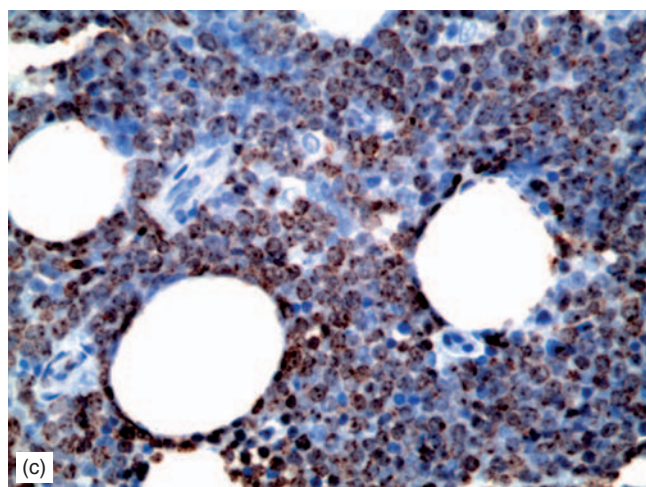


FIGURE 15.72 (Continued)

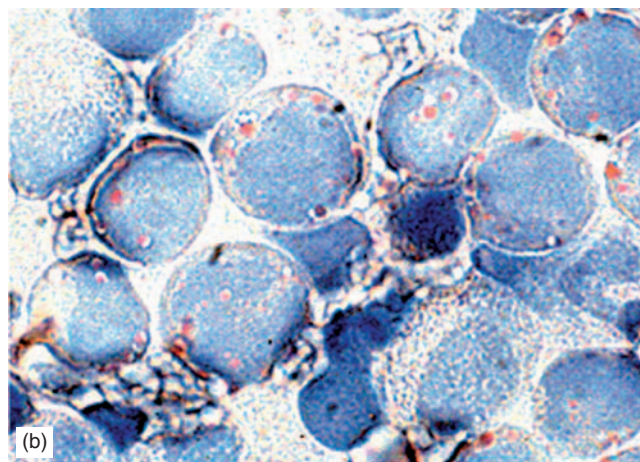
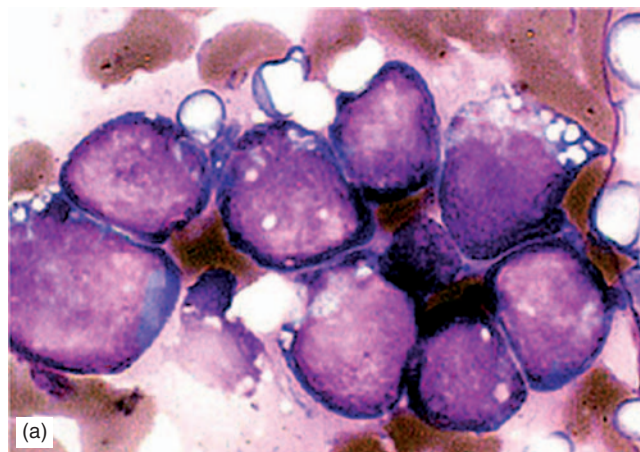
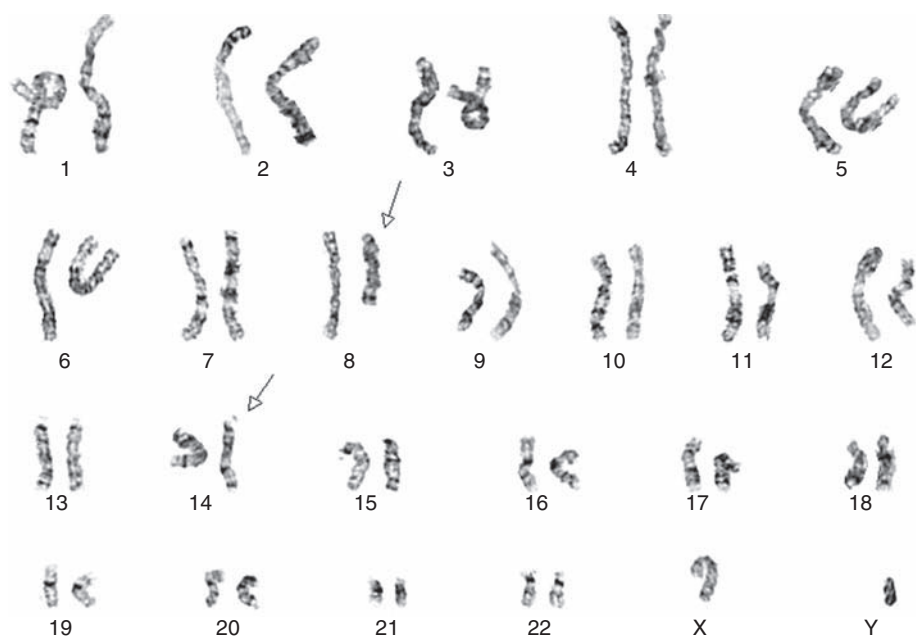
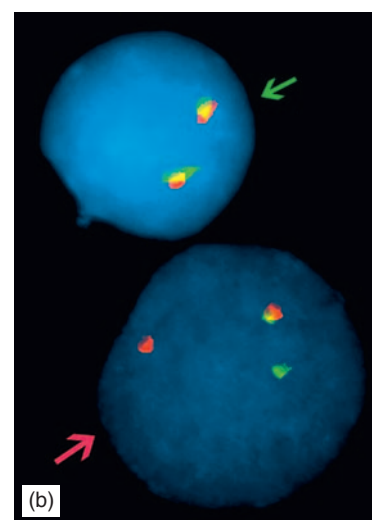


FIGURE 15.73 Touch preparation of a lymph node involved with Burkitt lymphoma. (a) Wright's stain demonstrates tumor cells with vacuolated cytoplasm. (b) Oil red O stain shows numerous orange-red dot-like deposits within the cytoplasmic vacuoles. From Ref. [91] by permission.



(a)



(b)

FIGURE 15.74 Burkitt lymphoma. (a) Karyotype demonstrating 46,XY,t(8;14)(q24;q32). (b) FISH analysis with split C-MYC signals.

distribution of endemic malaria. The disease usually presents as a mass in the jaw or facial bones and spreads to other extranodal sites, such as bone marrow, peripheral blood, meninges, testis, ovary, kidney, and breast.

Sporadic BL is seen all over the world accounting for about 1–2% of all lymphomas in the United States and Western Europe. It occurs mainly in children and young adults and represents 30–50% of all childhood lymphomas [1, 2]. The most common clinical presentation is abdominal mass with ascites involving stomach, distal ileum, cecum, and/or mesentery. Bone marrow, peripheral blood, kidney, testis, ovary, breast, and CNS may be involved.

Immunodeficiency-associated BL is primarily observed in patients with HIV infection. Post-transplant-associated BL is less frequent. This form more often involves lymph nodes but may involve bone marrow and peripheral blood and presents as acute leukemia.

Rapidly growing mass with elevated serum LDH levels are characteristic features of BL. Approximately 70% of patients are in advanced stages of the disease at diagnosis. BL is considered as a highly aggressive tumor. Involvement of the bone marrow and CNS, tumor size >10 cm in diameter, and elevated serum LDH are considered poor prognostic factors. Response to intensive combination chemotherapy with complete remission approaches 90% in early stages and 60–80% in advanced stages of the disease. Allogeneic bone marrow transplantation has been utilized in patients not responding to chemotherapy [2, 333, 334].

Differential Diagnosis

The differential diagnosis includes precursor B acute lymphoblastic leukemia/lymphoma and DLBCL. Precursor B-cell neoplasms are often TdT- and CD34-positive and lack the expression of membrane Ig heavy or light chains, whereas BL cells are negative for CD34 and TdT and express membrane Ig. Some cases of BL are borderline and share some of the morphologic features of DLBCL by demonstrating larger cells or an admixture of centroblast- or immunoblast-like cells. Also, a minority of DLBCL cases may demonstrate t(8;14) and C-MYC deregulation. The following recommendations are provided for the distinction of BL from DLBCL in borderline cases (Table 15.10) [277, 335]:

1. In tumors with borderline morphologic features, diagnosis of BL is made when the Ki-67-positive fraction is at least 99% in viable tumor cells.
2. Tumors with typical morphologic features of DLBCL with high Ki-67 fraction or t(8;14) and morphologically borderline cases with low Ki-67 fraction should be classified as DLBCL.

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Plasma Cell Myeloma and Related Disorders

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GENERAL CONSIDERATIONS

The monoclonal proliferation of plasma cells, also known as “monoclonal gammopathies,” “plasma cell dyscrasias,” or “paraproteinemias,” consists of several clinicopathologic entities. It is characterized by a single class of immunoglobulin (Ig) product, referred to as “M-component” (monoclonal component), present in serum and/or urine protein electrophoresis (Figure 16.1) [1–3]. In addition to protein electrophoresis, other more sensitive and specific techniques, such as immunoelectrophoresis and immunofixation, have been used to detect the specific class or subtype of monoclonal Ig in these disorders (Figures 16.2 and 16.3).

The following categories are discussed in this chapter. Waldenstrom macroglobulinemia (lymphoplasmacytic lymphoma) has already been discussed in Chapter 15.

Monoclonal gammopathy of undetermined significance (MGUS)

Plasma cell myeloma (PCM) and variants

Plasmacytoma and variants

Monoclonal Ig deposit diseases

Heavy chain diseases.

Etiology and Pathogenesis

The etiology of these related disorders is not known. Several environmental and genetic factors have been associated with the increased risk of PCM. Increased incidence of PCM has been observed in the Japanese survivors of atomic bomb and in radiologists exposed to long-term low doses of radiation [4–6]. Occupations in

agriculture and metal industries and exposure to benzene and hair dyes have been reported to be in association with increased risk of PCM [7–9]. There are also reports linking obesity and diet to the increased risk of PCM [10, 11].

There are several reports of familial clusters of PCM affecting two or more first-degree relatives suggesting that hereditary factors may be involved in the development of monoclonal gammopathies in certain conditions [12–14]. Viral infections, such as hepatitis C, HIV, and HHV-8 (Kaposi-sarcoma-associated herpes virus), have been associated with increased risk of PCM [15–17].

Dysregulation in immune function may also play a role in the pathogenesis of monoclonal plasma cell disorders [18–20].

Molecular genetic studies have shed some light on the pathogenesis of these disorders (Figure 16.4) [21, 22]. A broad spectrum of abnormalities have been identified in the signaling pathways, cell cycle, apoptotic processes, and bone marrow microenvironment. For example, the monoclonal plasma cells are capable of producing IL-6 and IL-6 receptor, leading to autocrine stimulation [23–25]. IL-6 activates two pathways: the *JAK2-STAT3* and the *RAS-MAP*. The first pathway upregulates anti-apoptotic proteins Mcl-1 and Bcl-X, and the second pathway leads to the upregulation of transcription factors, such as ELK-1 and AP-1 [25–28].

Gene expression profiling studies have identified three genes which may play important roles in pathogenesis and clinical outcome of PCM: *RAN*, *CHC1L*, and *ZHX-2* [29]. The *RAN* gene maps to 12q24, is a member of the Ras family, and has a role in regulating chromosome condensation, spindle formation, and cell-cycle progression. The *CHC1L* gene maps

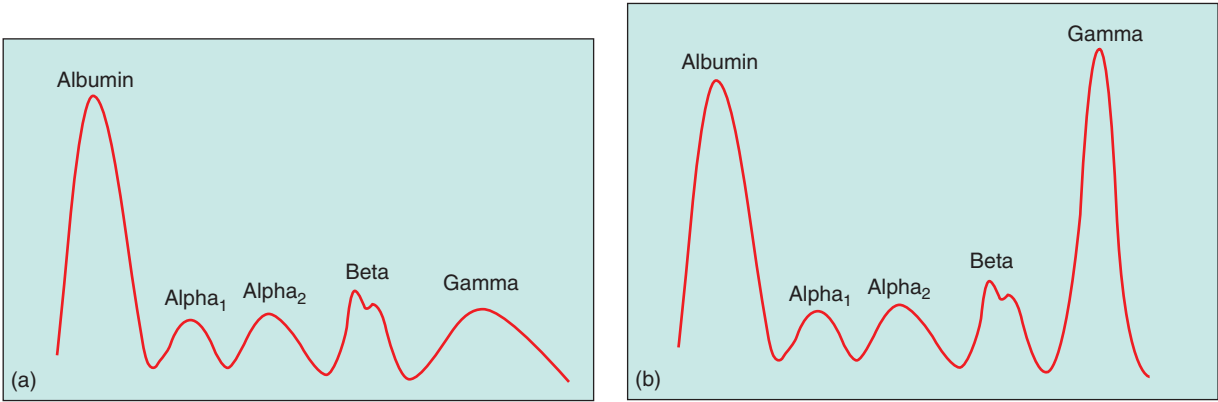


FIGURE 16.1 Schematics of serum protein electrophoresis: (a) demonstrates a normal profile and (b) shows a spike in the gamma region.

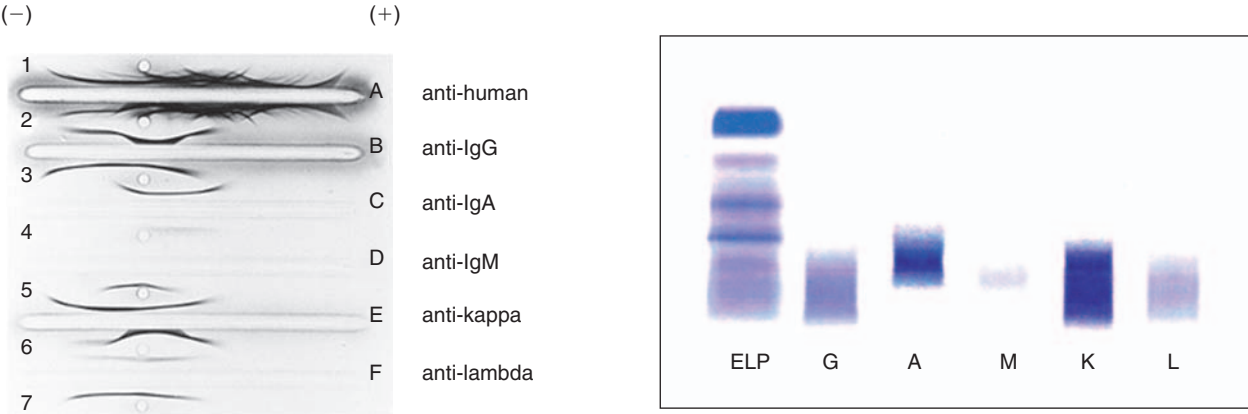


FIGURE 16.2 Serum immunoglobulin electrophoresis with a panel of IgG, IgA, IgM, kappa, and lambda antibodies demonstrating an IgG/kappa spike. Wells 1–7 contain control serum (odd number) and patient serum (even number). From Bossuyt X, Bogaerts A, Schiettekatte G, Blanckaert N. (1998). Serum protein electrophoresis and immunofixation by a semiautomated electrophoresis system. *Clin Chem* **44**, 944–9.

FIGURE 16.3 Immunofixation analysis of a serum demonstrating IgA-kappa monoclonal gammopathy. Courtesy of Eugene Dinovo, Ph.D., Department of Veterans Affairs, Greater Los Angeles Healthcare System.

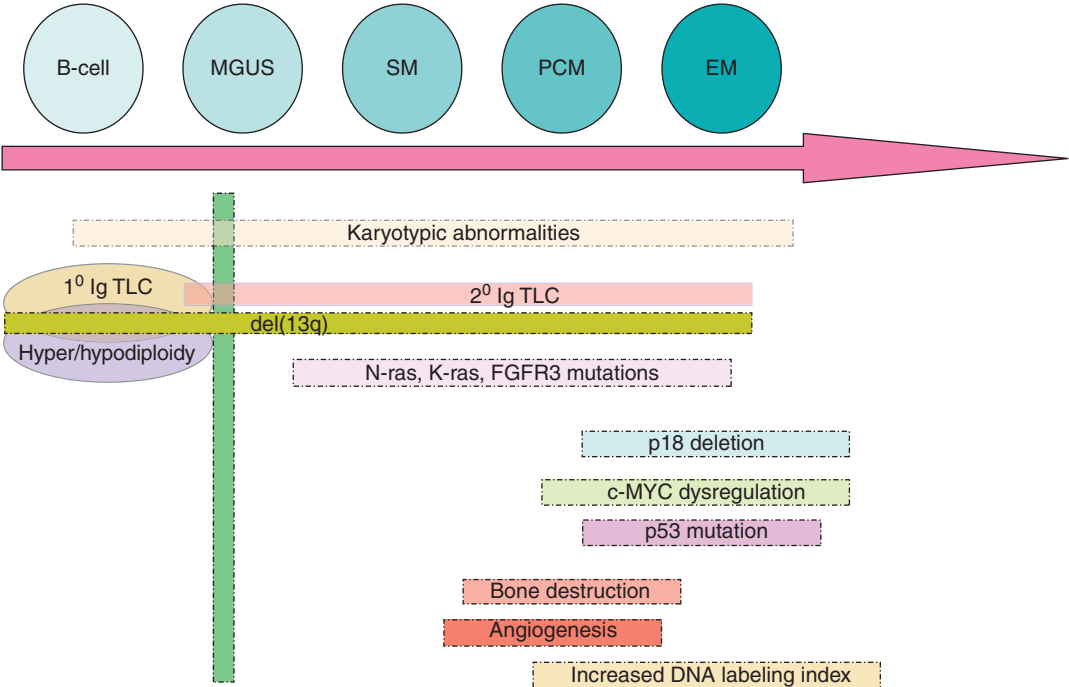


FIGURE 16.4 Schematic stages of molecular, cytogenetic, and clinical events in plasma cell disorders from monoclonal gammopathy with undetermined significance (MGUS) to smoldering myeloma (SM), plasma cell myeloma (PCM), and extramedullary myeloma (EM). Adapted from Ref. [21].

to 13q14.3 and appears to harbor a tumor suppressor gene for PCM. The *ZHX-2* gene maps to 8q24, near the *MYC* oncogene, and is a negative regulator of the NF- κ B transcription factor. This factor is a master transcriptional regulator of numerous genes involved in cell-cycle control and proliferation.

The levels of cyclin D1, cyclin D2, or cyclin D3 mRNA in the tumor cells of almost all patients with MGUS and PCM are relatively high. Increased expression of one of the cyclin D proteins facilitates activation of CDK4, leading to inactivation of the *RB1* (retinoblastoma 1) gene, and consequently cell-cycle progression [21].

Pathology

Morphology

The morphologic hallmark of PCM and related disorders is an increase in bone marrow plasma cells. The neoplastic plasma cells have a tendency to appear in clusters or sheets in the biopsy sections and, unlike reactive plasmacytosis, are not located around the vascular structures (Figure 16.5). The bone marrow biopsy sections are usually hypercellular, but occasionally they may appear hypocellular (Figure 16.6). Prominent osteoclastic activity may be evident, adjacent to the bony trabeculae.

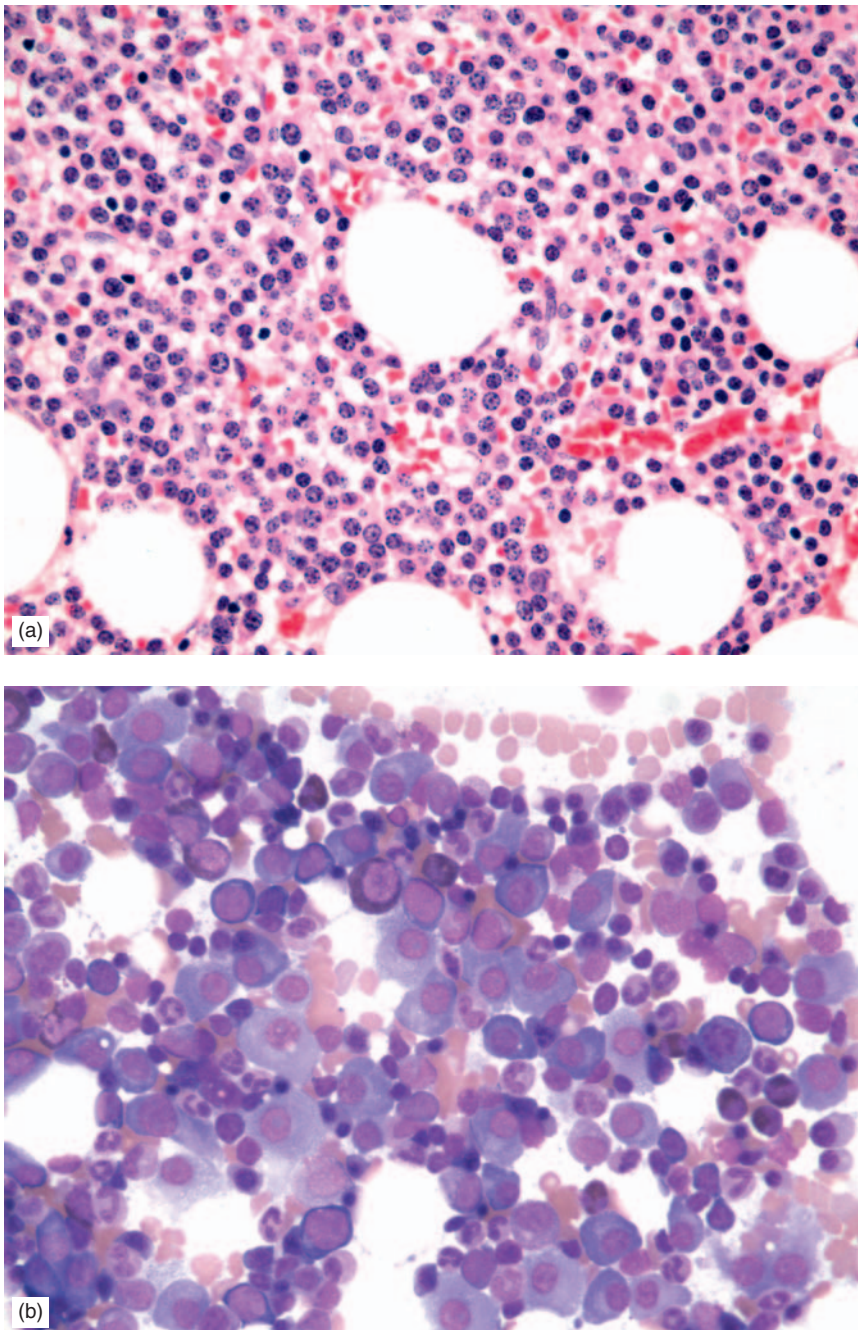


FIGURE 16.5 Plasma cell myeloma. Bone marrow biopsy section (a) and bone marrow smear (b) demonstrating numerous plasma cells.

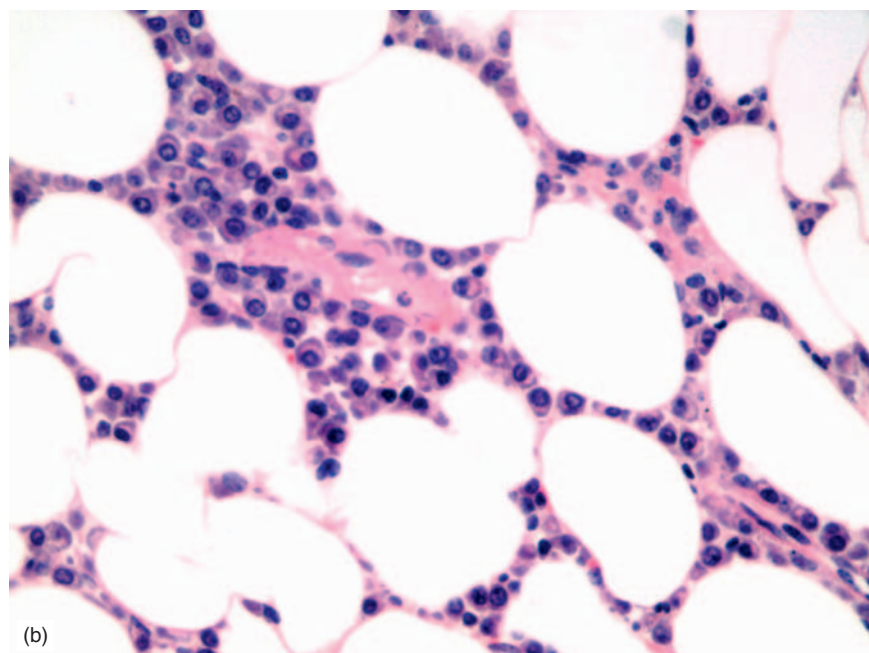
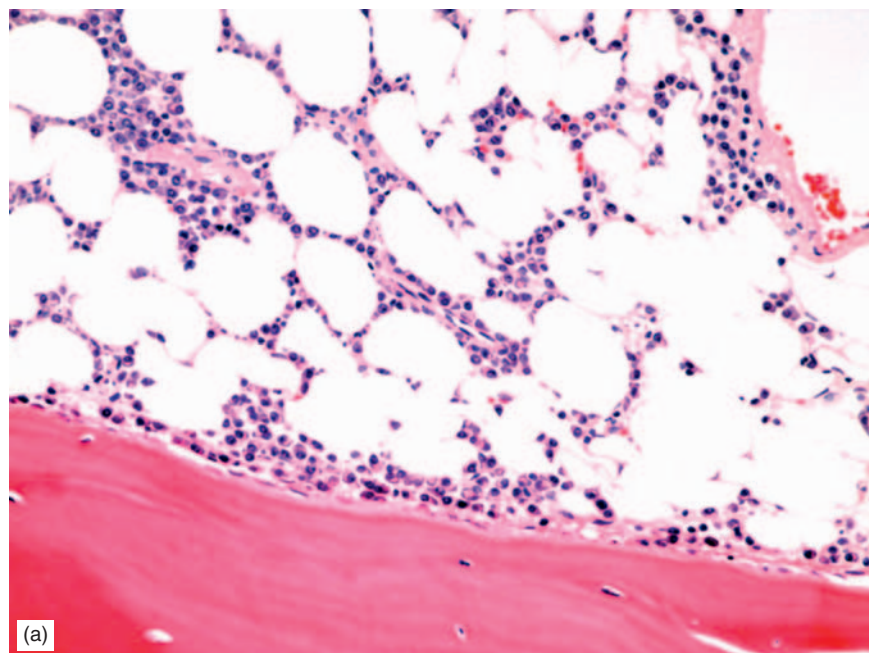


FIGURE 16.6 Hypocellular plasma cell myeloma with interstitial infiltration of plasma cells around fatty tissue: (a) low power and (b) high power views.

The cytologic features of plasma cells in the bone marrow smears may vary from normal-appearing mature plasma cells to immature and anaplastic forms (Figures 16.7–16.9). Plasmablasts show a high nuclear:cytoplasmic ratio, deep blue cytoplasm, with or without perinuclear hof, round or irregular nucleus, fine chromatin, and one or several prominent nucleoli (Figure 16.9). Multinucleated or multilobated plasma cells may be present. Cells with cherry-red, round cytoplasmic (Russell bodies) or nuclear (Dutcher bodies) inclusions, as well as cytoplasmic crystals may be present (Figures 16.10 and 16.11). Some plasma cells may appear like grapes and demonstrate numerous, round, Ig-containing cytoplasmic structures (Mott and Morula cells) (Figure 16.12). The blood

smears may show rouleaux formation of the red blood cells or the presence of plasma cells in various proportions (Figure 16.9c). Solitary neoplasms of plasma cells (plasmacytoma) may involve bone or other extramedullary sites.

Immunophenotype

Plasma cells in myeloma and related disorders, similar to normal plasma cells, lack surface Ig and express cytoplasmic Ig, CD38, CD138, and CD79a. CD138 is more specific than CD38 in the detection of plasma cells (Figures 16.13 and 16.14) [30–34]. However, the CD138 molecules disintegrate and disappear fast and therefore may not be detected

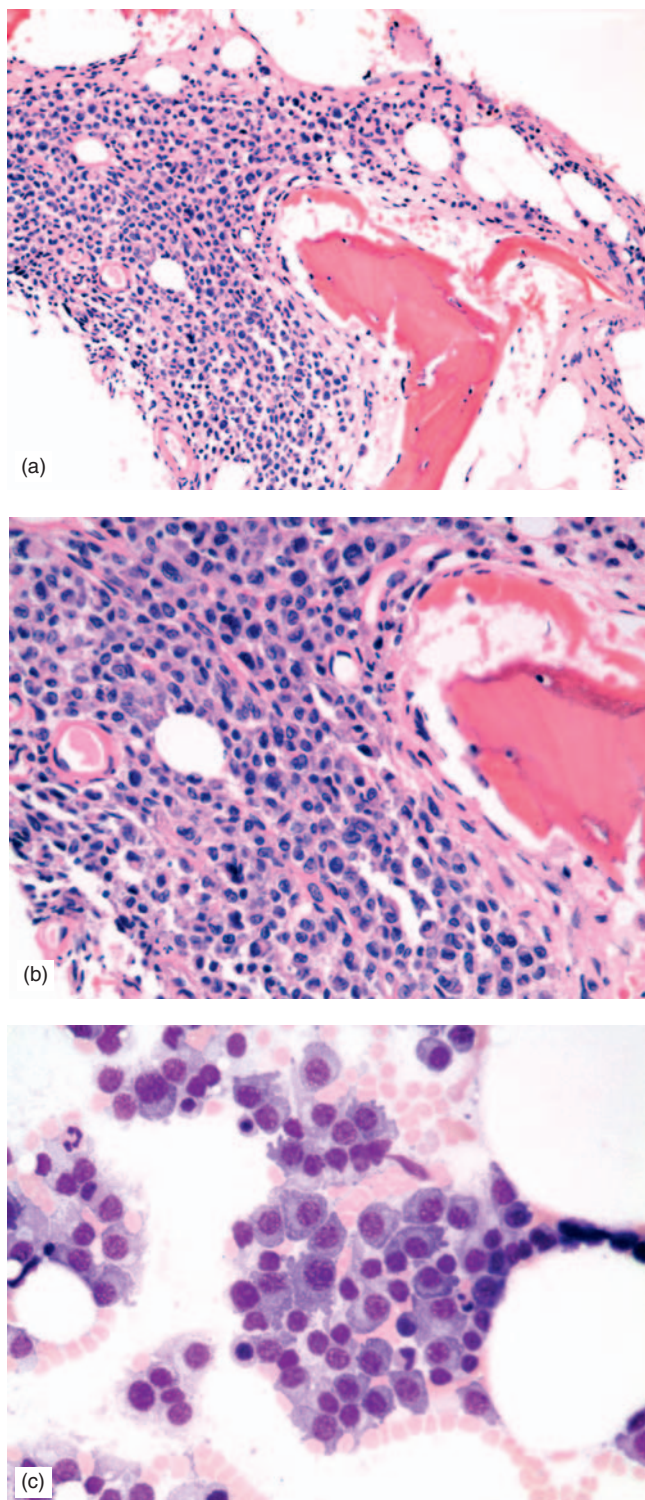


FIGURE 16.7 Plasma cell myeloma. Bone marrow biopsy section demonstrating a paratrabecular aggregate of plasma cells: (a) low power and (b) high power views. Bone marrow smear shows clusters of plasma cells (c).

by flow cytometry if the specimen is not prepared immediately. Also, most flow cytometry laboratories have experienced a significant loss in the number of plasma cells during the cell preparation for the flow cytometry procedures.

Therefore, if the percentage of plasma cells in the bone marrow smears is not high, immunohistochemistry on the bone marrow biopsy sections is recommended.

Plasma cells in PCM and related disorders are monoclonal and express only one type of Ig light chain. The class of Ig is usually IgG, less frequently IgA, and occasionally IgD, IgE, or IgM. The neoplastic plasma cells often lack CD19 and CD20 expressions but may aberrantly express CD10, CD56, or, less frequently, myelomonocytic or T-cell-associated markers [30–34]. Both normal and neoplastic plasma cells may express CD117.

Cytogenetic and Molecular Studies

PCM is a genetically unstable malignancy of post-germinal center B-lineage cells. Chromosome analysis in PCM is hampered by the low proliferative fraction in most cases. Recent studies using cytokine-stimulated bone marrow cultures and FISH and microarray techniques have increased the proportion of informative cases.

Active myeloma is often preceded by an indolent phase of MGUS, where the plasma cells are already abnormal with an aneuploid DNA content. In fact, almost all myeloma tumors and most cases of MGUS are aneuploid as demonstrated by DNA content measurements using flow cytometry, conventional cytogenetics, or molecular cytogenetics (FISH) [35, 36].

By classical cytogenetics, only one-third of PCM patients have a complex abnormal karyotype. The remaining two-thirds have normal karyotypes [37, 38]. However, the observed normal karyotypes are often derived from the other non-neoplastic hematopoietic cells, rather than from abnormal plasma cells, because the plasma cells fail to grow. Plasma cells may fail to grow in culture for three major reasons. First, samples from PCM patients may fail to grow because of the low proliferative capacity of the myeloma cells [39]. Myeloma cells (especially in early myeloma) are stroma dependent, so removing the cells from their supportive microenvironment results in apoptosis and lack of growth. However, if myeloma cells have become stroma independent (i.e. in advanced stages of the disease), removal of the myeloma cells from their microenvironment can result in proliferation and an abnormal karyotype [39]. The second explanation for the laboratory's inability to obtain abnormal metaphases lies in the quality of the bone marrow aspirates received for cytogenetic studies. Aspirates frequently contain drastically fewer plasma cells than the corresponding smears used for morphological assessment since the number of tumor cells in a given specimen largely depends on the level of local bone marrow infiltration and the degree of sample dilution by blood [38]. For this reason, it is essential that the first few milliliters of the bone marrow drawn be sent for cytogenetic analysis. Also, the needle should be repositioned during aspiration, rather than simply continuing to withdraw marrow from the initial puncture site, to ensure that adequate numbers of abnormal cells are submitted to the laboratory. Finally, aspirates should be processed as soon as possible if FISH is requested. Several techniques have been created to selectively culture plasma cells [40], but these methods may not be helpful if the sample provided to the laboratory is of poor quality.

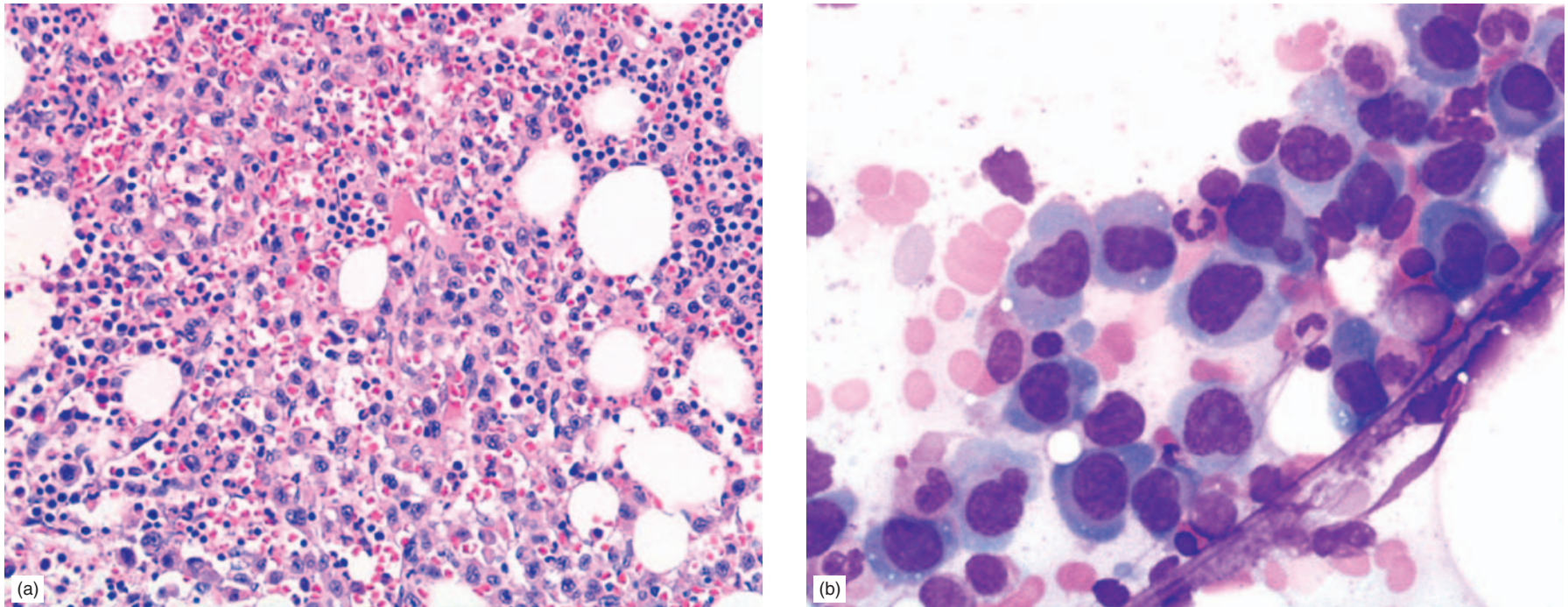


FIGURE 16.8 Plasma cell myeloma. Bone marrow biopsy section (a) and bone marrow smear (b) demonstrate numerous, large, atypical plasma cells with abundant cytoplasm, irregular nuclear borders, and prominent nucleoli.

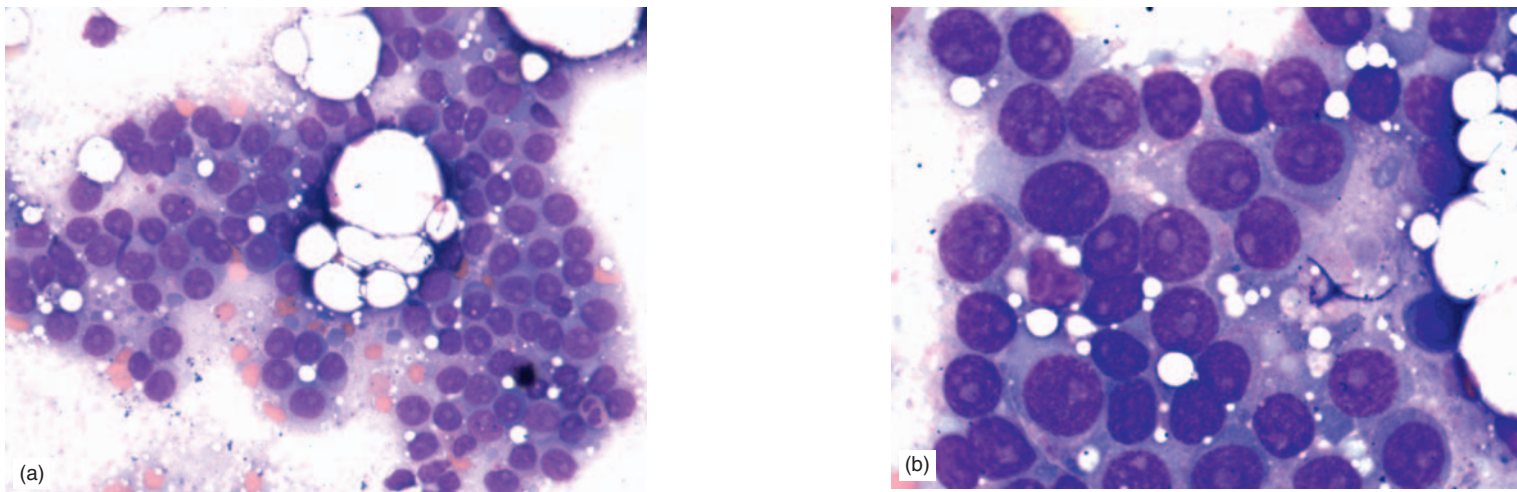


FIGURE 16.9 Plasmablastic myeloma. Bone marrow smear demonstrating cells with variable amounts of blue cytoplasm, round nuclei with finely dispersed chromatin, and a single prominent nucleolus: (a) low power and (b) high power views.

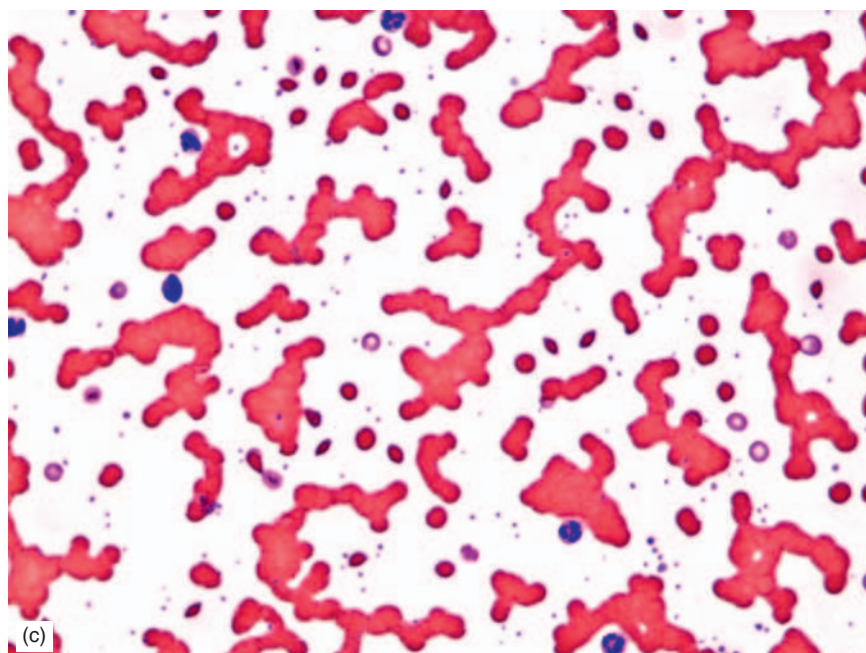
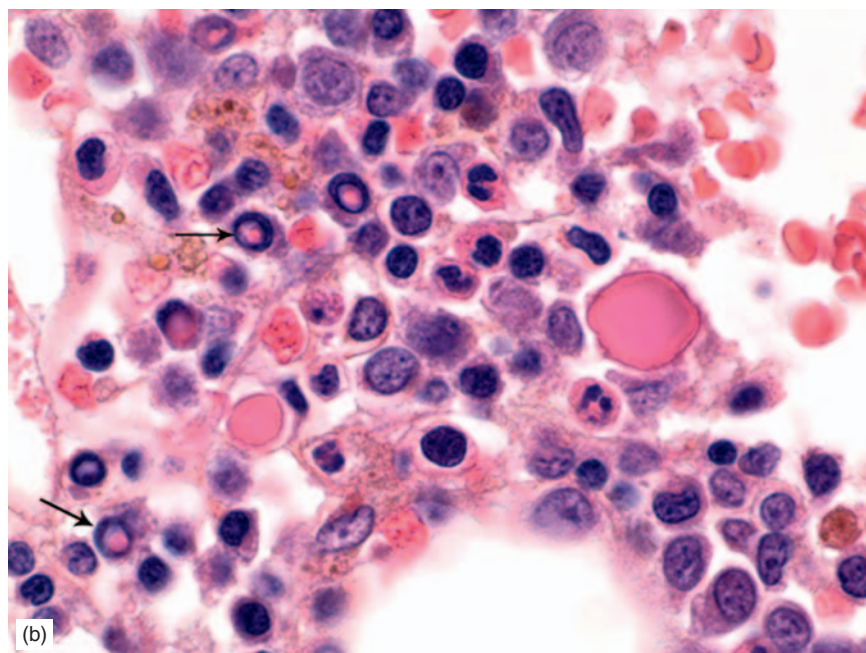
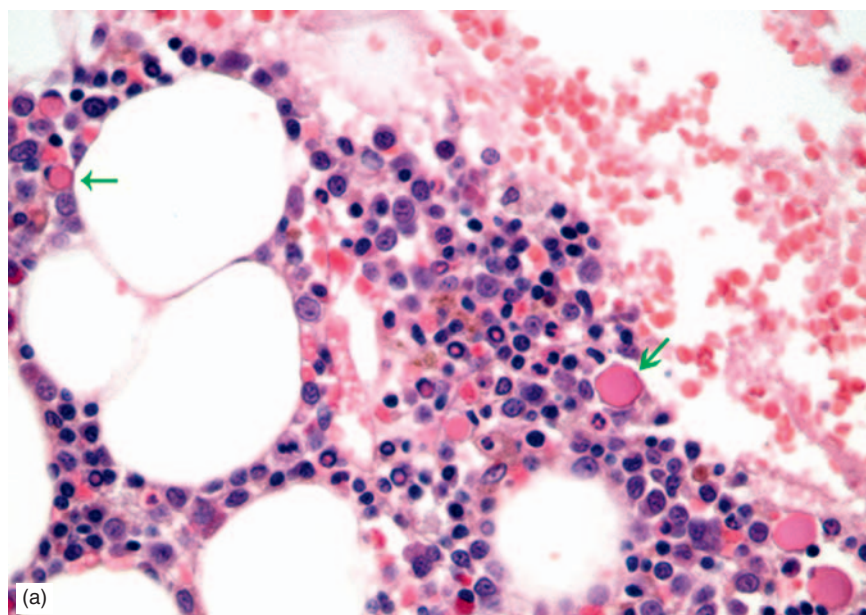


FIGURE 16.10 Plasma cell myeloma. Bone marrow biopsy section demonstrating plasma cells with nuclear inclusions (Dutcher bodies, black arrows) and cytoplasmic inclusions (Russell bodies, green arrows): (a) low power and (b) high power views. Blood smear showing formations (c).

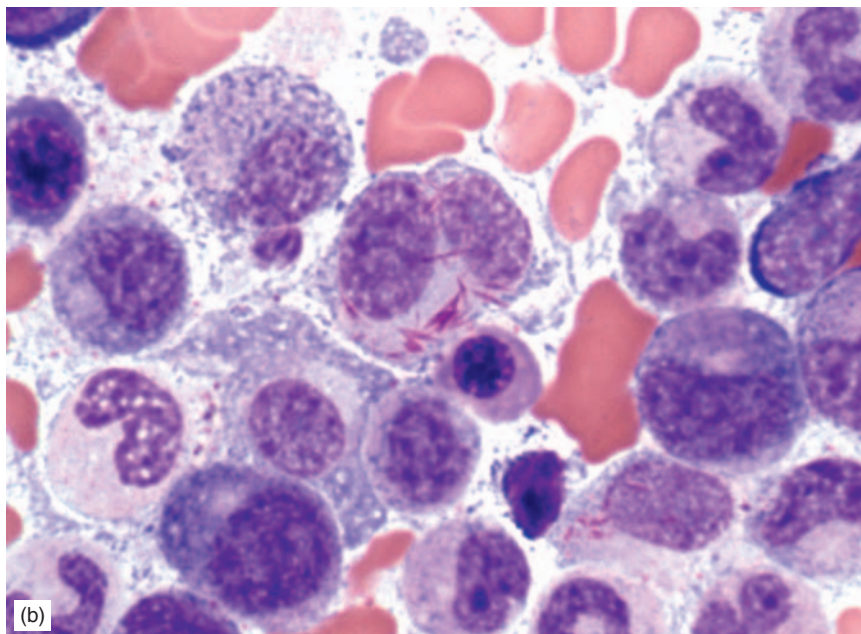
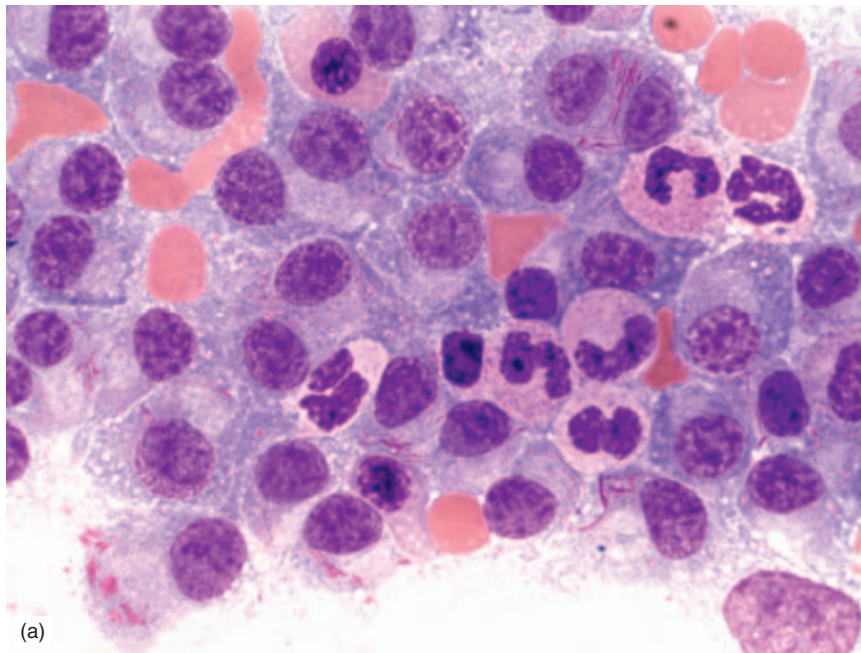


FIGURE 16.11 Plasma cell myeloma. Bone marrow smear demonstrating plasma cells with cytoplasmic needle-like immunoglobulin crystals: (a) low power and (b) high power views.

Several recurring aberrations are observed in karyotypically abnormal PCM. The majority of PCM cases demonstrate chromosomal aneuploidy. Four categories of aneuploidy can be defined by karyotyping:

1. Hypodiploidy (<46 chromosomes) (Figure 16.15).
2. Pseudodiploidy (46–47 chromosomes but with structural or numerical abnormalities).
3. Hyperdiploidy (>50 chromosomes, HRD) (Figure 16.16).
4. Near-tetraploidy (>75 chromosomes).

Multiple non-random trisomies are associated with hyperdiploid tumors [41], especially trisomies of

odd-numbered chromosomes [40]. It was initially believed that trisomies were more common than monosomies in PCM, but the opposite is true. The most common trisomies are of chromosomes 3, 5, 7, 9, 11, 15, 19, and 21 [42]. The most common monosomies are of chromosomes 13, 14, 16, and 22. No specific numerical chromosomal abnormality is constant or predictive of disease progression. The prevalence of aneuploidy is independent of stages. Karyotypes are typically complex and exhibit >10 abnormalities in almost half of the patients and even >20 aberrations in about 10% of the cases. HRD, present in nearly 60% of PCM, is most often associated with trisomies of chromosomes 3, 5, 7, 9, 11, 15, 19, and 21 and represents one of the central genetic

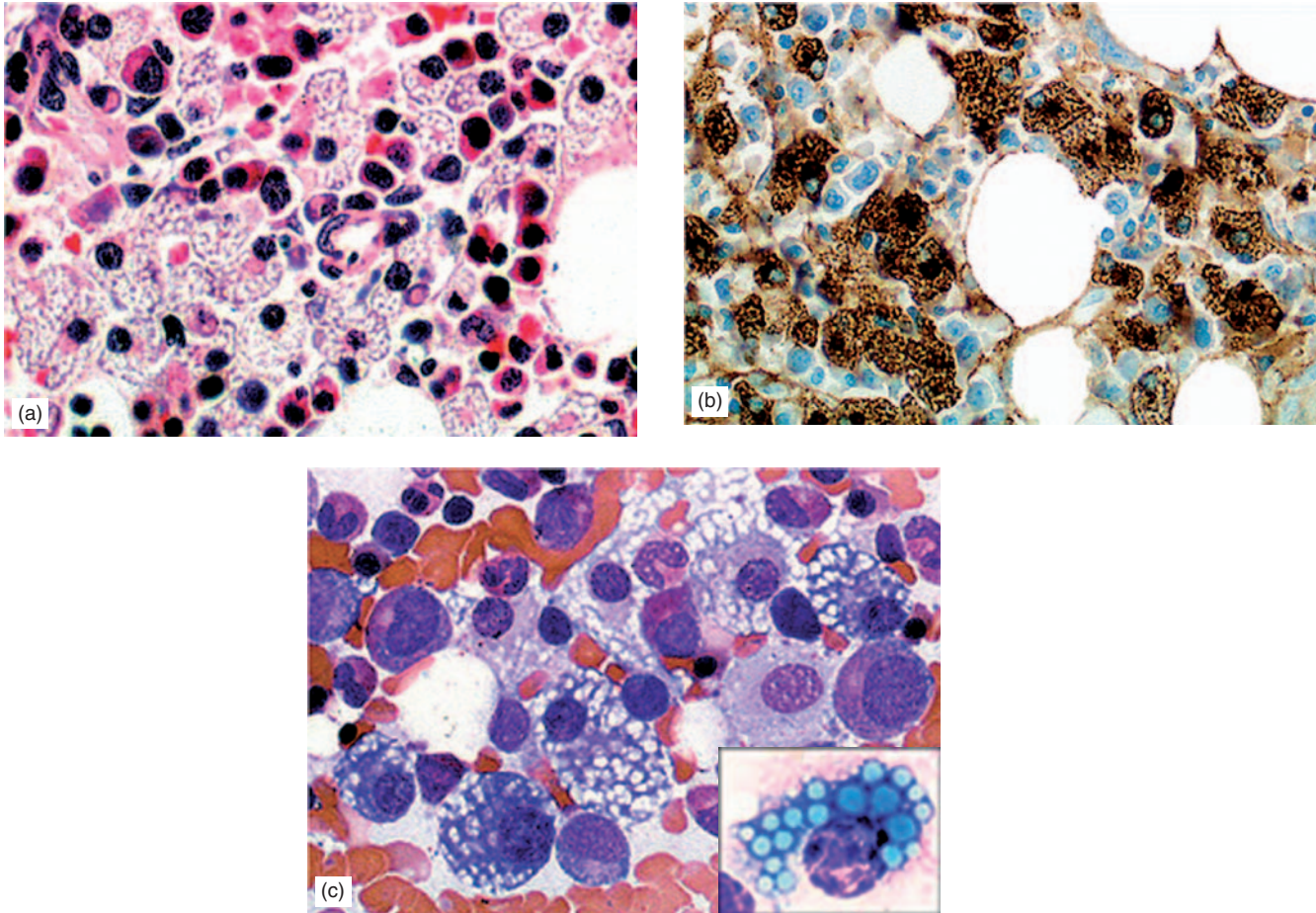


FIGURE 16.12 Plasma cell myeloma with cytoplasmic vacuoles. (a) Bone marrow biopsy section, (b) immunohistochemical stain for Ig kappa light chain, and (c) bone marrow smear. From Naeim F. (1997). *Pathology of Bone Marrow*, 2nd ed. Williams & Wilkins, Baltimore, by permission.

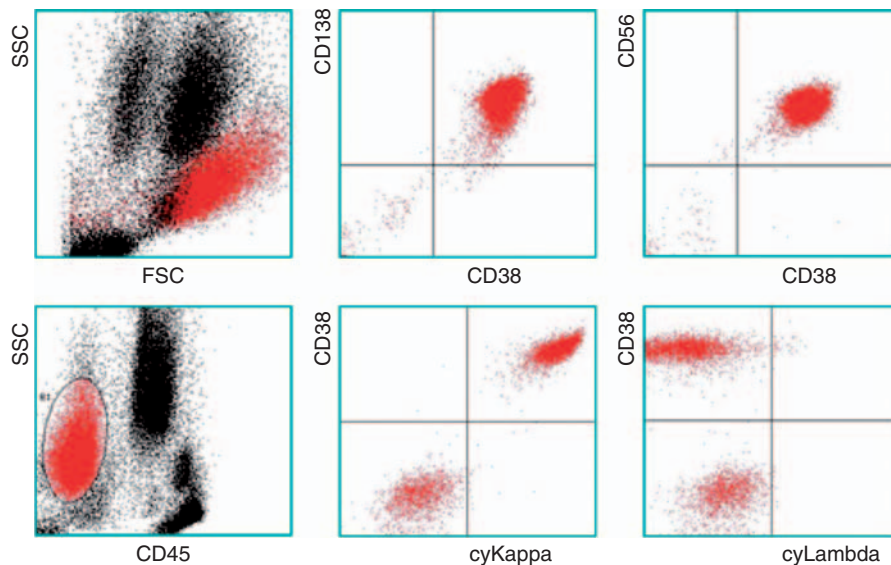


FIGURE 16.13 Flow cytometry of plasma cell myeloma demonstrating a population of cells (red) which are negative for CD45 and express CD38, CD138, CD56, and cytoplasmic kappa light chain.

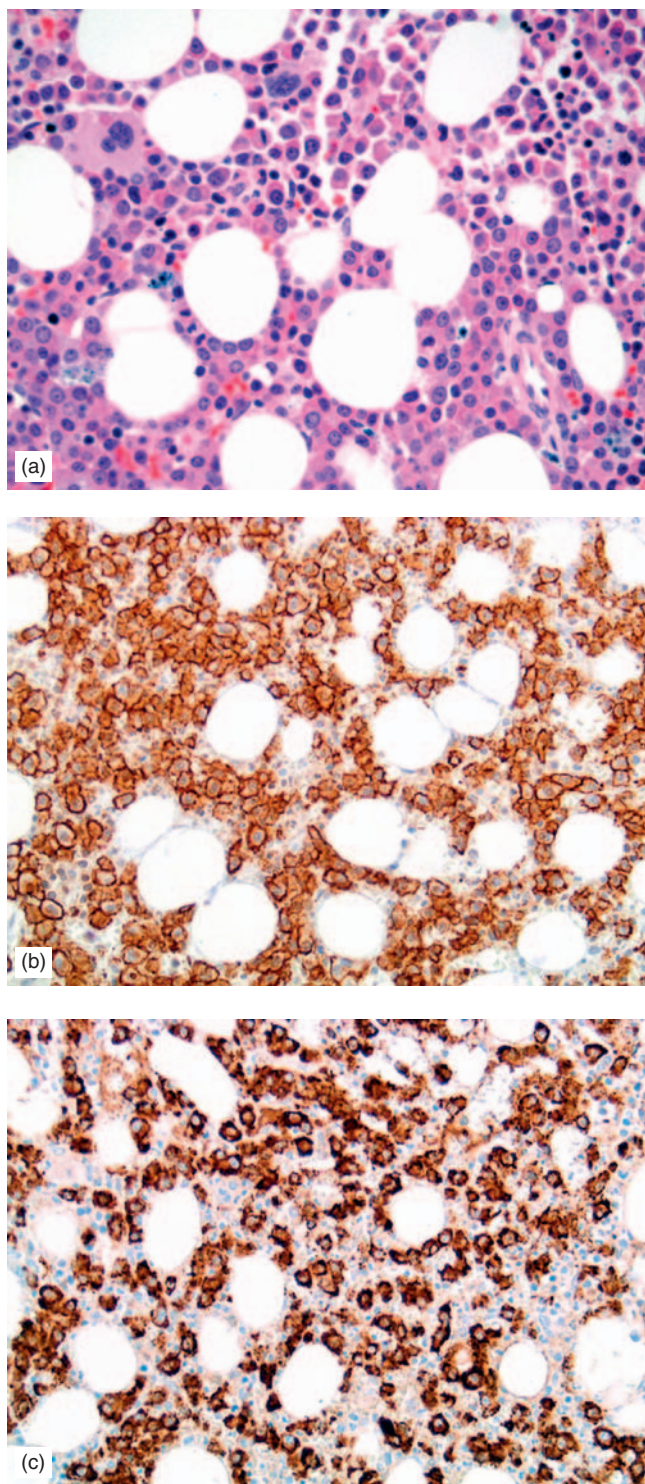


FIGURE 16.14 Plasma cell myeloma. Bone marrow biopsy section demonstrating a large population of plasma cells (a). Immunohistochemical stains show expression of CD138 (b) and kappa light chain (c) by the plasma cells.

pathways in the development of PCM, and this type of disease has been previously shown to have distinct gene expression signature [21]. Hyperdiploidy is seen in about 40% of PCM patients, with an incidence of 25% of the hypo- and

pseudodiploid karyotypes. Triploidy and tetraploidy are very rare, usually not observed over 10%. Although the reasons are still unclear, the frequency of HRD has been shown to be more common in elderly patients [43].

The vast majority of PCM involves the translocation of the *IGH* gene on 14q32 to one of several non-random partners (~40 gene regions) and this is considered to be an initial event in the genesis of PCM (seen in about 40% of patients) [40]. Standard cytogenetic analyses identify abnormalities of 14q32 in up to 40% of cases (Figures 16.17–16.19), while this detection rate nearly doubles with interphase FISH studies. Illegitimate recombination of the *IGH* gene also occurs in MGUS but at a slightly lower frequency (~50%). Similar rearrangements of the *IGK* and *IGL* genes have been found to occur in a small subset of PCM and MGUS [44]. The rearrangements involving the *IGH* gene are commonly simple reciprocal translocations, but more complex recombination events, such as insertions and duplications, are also observed (Tables 16.1 and 16.2).

Cyclin D1 (most common), D2, and D3 genes (*CCND1*, *CCND2*, and *CCND3*) on chromosomes 11q13, 12p13, and 6p21, respectively, MAF family member genes (*c-MAF*, *MAFA*, and *MAFB*) on chromosomes 16q23, 8q24, and 20q11, respectively, and the fibroblast growth factor receptor 3 gene (*FGFR3*) on chromosome 4p16 are commonly observed as *IGH* translocation partners [1]. These translocations are markers for distinct subtypes of myeloma with important prognostic implications. *IGH* gene translocations are found more frequently in non-HRD tumors (70%) than in HRD tumors (20%). The t(11;14) (Figure 16.16) and t(4;14) are the most common *IGH* translocations followed by t(14;16) (Figure 16.19), and t(14;20) is the least common [38, 40]. The t(14;16)(q32;q23) and t(14;20)(q32;q11.2) result in the activation of *c-MAF* and *MAFB* proto-oncogenes, respectively, and are together seen in approximately 6% of cases. The reciprocal t(4;14)(p16;q32) translocation results in the hyperactivation of both the *FGFR3* and *MMSET* genes. Two cyclin D family members are activated by translocations in PCM: cyclin D1 by the t(11;14)(q13;q32) in 17% and *CCND3* by t(6;14)(p21;q32) (Figure 16.18) in 2%. The overall rate of 14q32 translocations, however, significantly increases with disease progression and reaches up to 90% in advanced tumors and human myeloma cell lines. *IGH* translocation and HRD act similarly through the upregulation of one of the cyclins (D1, D2, or D3) [39, 41]. Translocations between the Ig heavy chain locus and *CCND1*, *CCND3*, *c-MAF*, *MAFB*, *FGFR3*, and *MMSET* represent recurrent genetic lesions in approximately 40% of PCM [45, 46].

Although numerical and gross structural changes can be diagnosed by karyotyping without difficulty, small interstitial deletions, partial genomic gains, and cryptic translocations (e.g. *IGH* translocations) can be easily overlooked due to the karyotype's limited spatial resolution. Modern molecular-based techniques, such as CGH and FISH, allow the detection of genetic abnormalities independent of proliferating cells. With these methods, chromosomal aberrations are found in >90% of patients with PCM and most (if not all) patients with MGUS. Considering that analyses with interphase FISH and molecular genetic techniques reveal

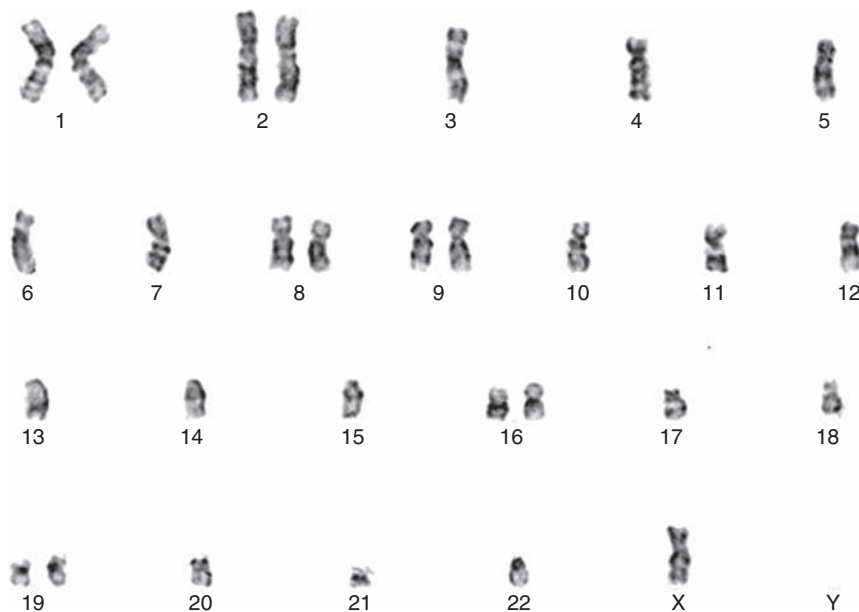


FIGURE 16.15 Bone marrow karyotype in a patient with plasma cell myeloma demonstrating hypodiploidy with 30,X,-X,-3,-4,-5,-6,-7,-10,-11,-12,-13,-14,-15,-17,del(17)(p13),-18,-20,-21,-22.

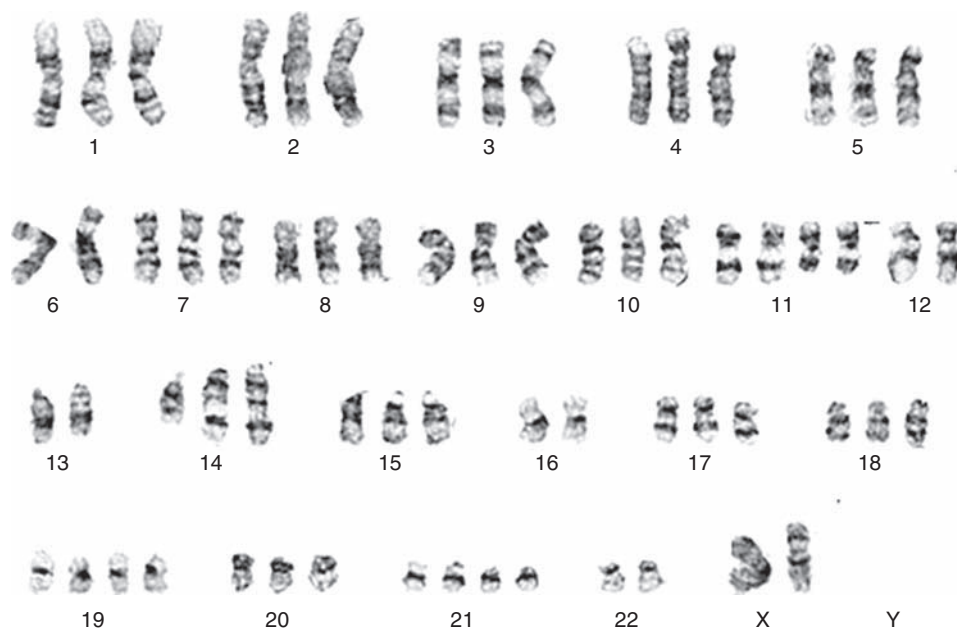


FIGURE 16.16 Bone marrow karyotype in a patient with plasma cell myeloma demonstrating hyperdiploidy with 66,XX,-X,-6,del(11)(q23),+del(11)(q23),-12,-13,der(14)t(11;14)(q13;q32)×2,-16,+19,+21,-22.

chromosomal abnormalities in close to 100% of PCM cases and in the majority of MGUS cases, the normal karyotypes are most likely not representative of the neoplastic clone.

FISH permits reliable identification of both translocations and small deletions or gains in PCM [4]. Most clinical laboratories currently test for 13q14 (*RB1*) and 17p13.1 (*TP53*) deletions as well as the primary translocations t(4;14)(p16.3;q32) and t(11;14)(q13;q32). Ploidy should be determined in all tumors; for example, disomy of chromosome band 13q14 in a near-tetraploid karyotype is functionally a deletion of the region. Polyploidy can be reliably excluded by the use of control probes mapped to genomic regions that rarely display aneuploidy (e.g. chromosomes 2, 10, and 12) [35, 37]. Finally, some laboratories test for t(6;14)(p21;q32), t(14;16)(q32;q23), and t(14;20), and

detection of the most frequent chromosomal abnormalities (e.g. +1q, +9q, +11q) [36].

Cytogenetics is helpful in determining myeloma patients' clinical outcome [36]. Normal metaphases and normal FISH, HRD tumors, and *CCND1* gene activation are associated with a better prognosis [1]. However, patients with *c-MAF*, *MAFB*, or *FGFR3* activation, del(13q), del(17p), hypodiploidy, 1q abnormalities, or 9q trisomies are associated with a worse prognosis [35, 39].

A strong association between chromosome 1q abnormalities and the etiology of PCM disease has been suggested. Tandem duplications and jumping translocations of 1q21 occur frequently in this malignancy [46]. The gains of 1q is one of the most common abnormalities in PCM [42]. In addition, hyperdiploidy with 1q was found to have a less



FIGURE 16.17 Bone marrow karyotype in a patient with plasma cell myeloma demonstrating 46,XY,t(13;14)(q14;q32).

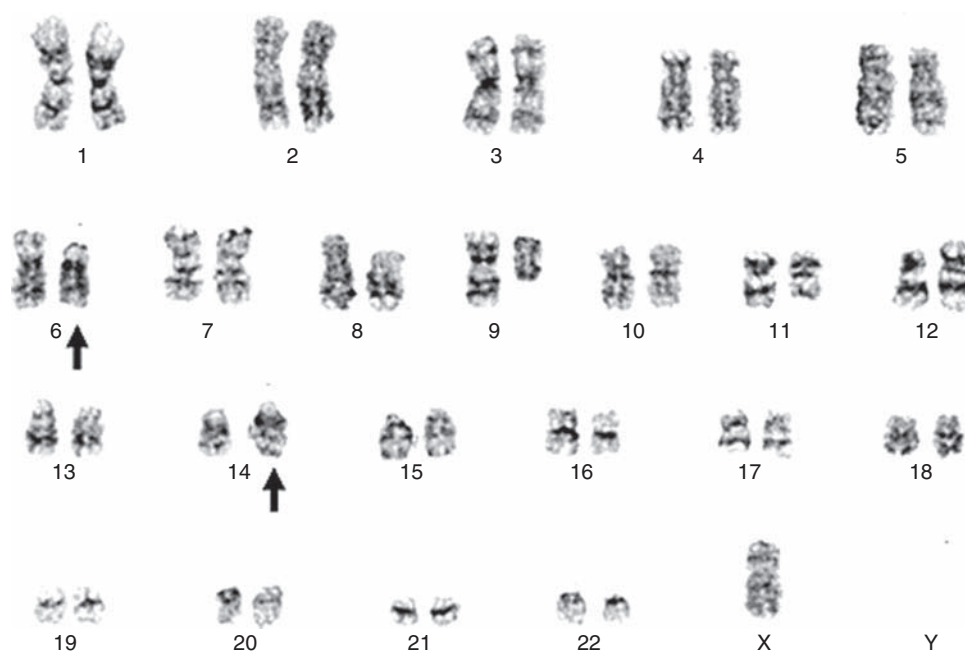


FIGURE 16.18 Bone marrow karyotype in a patient with plasma cell myeloma demonstrating 45,X,-Y,t(6;14)(p21;q32),iso(9)(q10),t(11;12)(q21;p13).

favorable clinical outcome than hyperdiploidy lacking this feature [47].

Additional copies of 1q21 have been reported to accompany the progression of smoldering to overt PCM by molecular and FISH studies such as array-CGH (aCGH) and interphase FISH [48]. Also it has been noted that patients with gains or amplifications of 1q21 were linked to inferior survival, and thus these events may be linked to PCM pathogenesis and progression.

Among losses, monosomy or partial deletion of chromosome 13 (13q14) is the most common finding, occurring

in 15–40% of the newly diagnosed cases (Figure 16.20). Patients with a 13q14 deletion have been reported to have significant reductions in the rate of response to conventional dose chemotherapy (41% versus 79%) and overall survival (24 versus >60 months) compared to patients without this deletion. When present, it was the most important independent variable associated with unfavorable outcome [49].

PCM and related disorders show clonal Ig gene rearrangements and high frequency of Ig VH gene somatic mutation. Approximately 5% of the cases of PCM may show more than one rearranged Ig bands best seen by Southern



FIGURE 16.19 Bone marrow karyotype in a patient with plasma cell myeloma demonstrating 45,XX,t(2;11)(p11.2;p11.2),-10,t(14;16)(q32;q23).

TABLE 16.1 Chromosomal regions/genes as translocation partners with 14q32(IGH) in PCM/MGUS with known frequencies.

4p16	<i>FGFR3,WHSC1</i> (15%)
4p13	<i>ARHH</i>
6p23-25	<i>IRF4</i>
6p21	<i>CCND3</i> (15–20%)
8q24	<i>c-MYC</i>
11q13	<i>CCND1</i>
16q23	<i>MAF</i> (5–10%)
18q21	<i>BCL2</i>
20q11-q13	<i>MAFB</i>

TABLE 16.2 Frequency of *IGH* translocation and clinical outcome.

Abnormality	PCM and smoldering PCM (%)	PCM (%)	Prognosis
<i>IGL</i> translocations	<20	<20	Unknown
<i>IGH</i> translocations			
t(4;14)	35–50	50–70	Poor
t(11;14)	2–10	15	Poor
t(14;16)	15–30	15	Good
t(6;14)	2–5	5	Poor

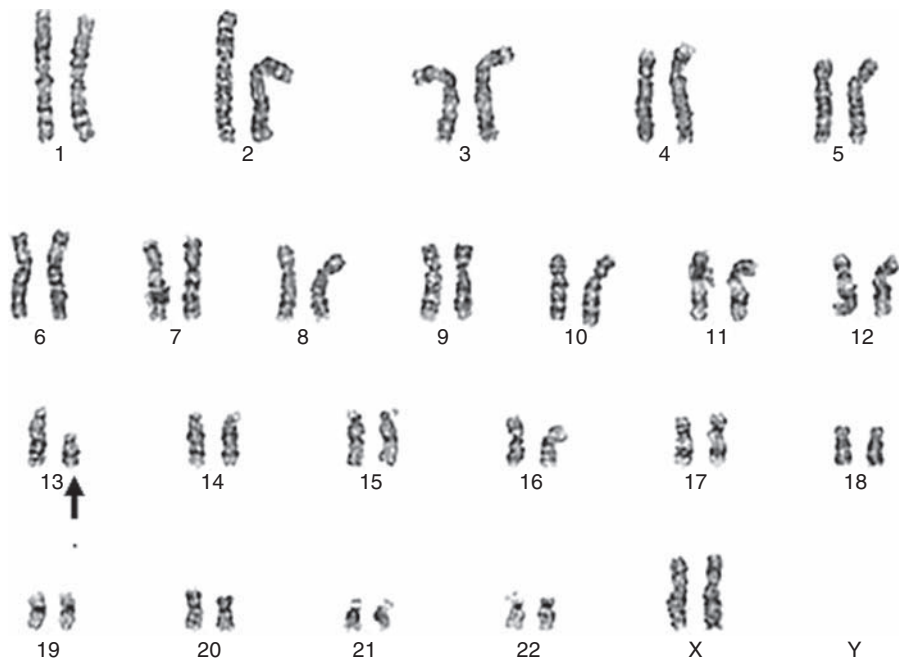


FIGURE 16.20 Bone marrow karyotype in a patient with plasma cell myeloma demonstrating 46,XX,del(13)(q13q22).

TABLE 16.3 Clinicopathological features of MGUS according to the International Myeloma Working Group.*

1. Serum M-protein level > 3 g/dL
2. Bone marrow monoclonal plasma cells >10%
3. No evidence of other B-cell lymphoproliferative disorders
4. No evidence of organ or tissue impairment or bone lesions

*From Ref. [51].

blot [1]. Approximately 50% of the tumors carry the translocation of Ig heavy chain (*IGH*) locus (14q32) with various oncogenes and suppressor genes, such as *cyclin D1*, *cyclin D3*, *PGFR3*, *CHC1L*, *ZHX-2*, *MAF*, and *MAFB* at 11q13, 6p21, 4p16, 13q14.3, 8q24.3, 16q23, and 20q12, respectively [50]. Because the diagnosis of myeloma is straightforward, such gene rearrangement studies are usually not required. However, detection of clonality by PCR may be helpful for monitoring minimal residual disease.

The importance of cytogenetic and molecular features as determinants of outcome is being increasingly recognized. The present data support that PCM is characterized by marked inter- and intratumor cytogenetic heterogeneity that may account for the diverse clinical behavior of this neoplasm. Multivariate analysis of all cytogenetic and clinicopathologic features, including patient survival, is needed to identify the cytogenetic variables of independent prognostic significance and also to define the predictive role of the proposed cytogenetic classification for response to treatment and survival of patients with PCM.

MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

The term “monoclonal gammopathy of undetermined significance” (MGUS) stands for a clinical condition defined by the presence of monoclonal Ig production without evidence of PCM, amyloidosis, Waldenström macroglobulinemia, or other related plasma cell or lymphoproliferative disorders (Table 16.3) [51]. Several other terms have been used for this condition, such as idiopathic, asymptomatic, non-myelomatous, cryptogenic, and benign monoclonal gammopathy. However, the term “benign monoclonal gammopathy” is inappropriate, because a significant proportion of patients with MGUS eventually develop PCM or other lymphoproliferative disorders. The cumulative probability of progression of MGUS to PCM or other lymphoproliferative disorders in one large study was 12% at 10 years, 25% at 20 years, and 30% at 25 years [52, 53]. Clearly, MGUS and PCM represent different time points along the same disease spectrum, and so far, no molecular or cytogenetic test can reliably distinguish them.

The incidence of MGUS increases by age. In a recent study, the prevalence of MGUS was 3.21% in individuals older than 50 and 5.3% in those older than 70 years [54]. It affects more African Americans than Caucasians. MGUS is usually an incidental finding detected by elevated total

protein concentration on a routine blood test, followed by demonstration of a monoclonal spike by serum protein electrophoresis. The presence of light chain in the urine (Bence-Jones protein) is generally suggestive of PCM. The nature of serum M-component in MGUS is IgG in about 75%, IgM in 15%, and IgA in 10% of the cases. Plasma cells in bone marrow biopsy sections and smears appear mature and account for <10% of the total bone marrow nucleated cells (Figure 16.21). Immunophenotypic studies reveal a monoclonal population expressing Ig molecules corresponding to the patient’s serum M-component and often an abnormal population of CD19– and CD56+ plasma cells. The elevated levels of monoclonal protein, presence of IgA or IgM class or an abnormal free light chain ratio, and a high percentage of plasma cells are predictors of MGUS progressing to a more aggressive B-cell lymphoproliferative disorder [55].

PLASMA CELL MYELOMA

Plasma cell myeloma (PCM) (multiple myeloma, myelomatosis, Kahler’s disease) is a multifocal bone-marrow-based plasma cell neoplasm with the production of monoclonal Ig, often associated with bone destruction and osteolytic lesions, hypercalcemia, and anemia [1, 51]. PCM has been divided into several clinicopathologic entities:

- Asymptomatic myeloma (smoldering myeloma)
- Indolent myeloma
- Symptomatic myeloma (or symptomatic plasma cell myeloma)
- Non-secretory myeloma
- Plasma cell leukemia.

Asymptomatic (Smoldering) Myeloma

Smoldering myeloma represents the point of transition from MGUS to PCM without anemia, skeletal lesions, hypercalcemia, or renal insufficiency. The serum M-protein level is ≥3 g/dL and the bone marrow plasma cells are ≥10% but <30% (Table 16.4). These patients do not need treatment but should be followed up closely, because many of them eventually become symptomatic.

Indolent Myeloma

This category is described by the WHO but not included in the report of criteria for the classification of plasma cell disorders by the International Myeloma Working Group (IMWG) [51]. According to the WHO and Alexanian [1, 56], indolent myeloma is similar to smoldering myeloma in that there is no evidence of anemia, hypercalcemia, or renal insufficiency, but unlike smoldering myeloma there are up to three lytic bone lesions and the serum M-component is at intermediate levels (IgG >3 and <7 g/dL).

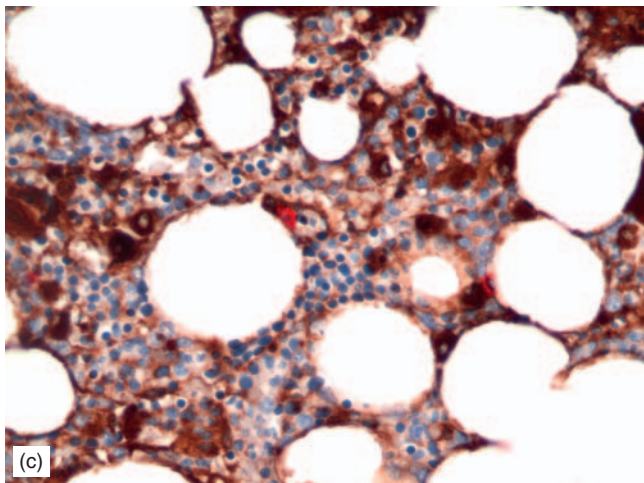
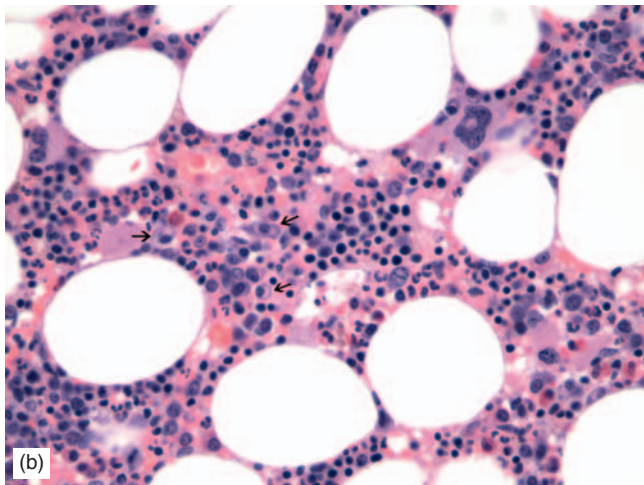
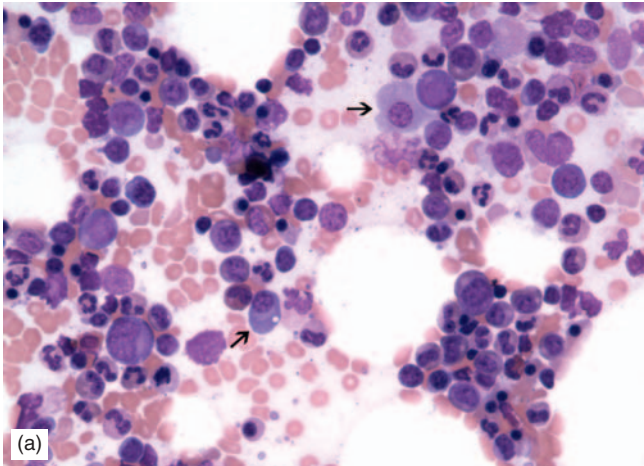


FIGURE 16.21 Monoclonal gammopathy of undetermined significance. Bone marrow smear (a) and biopsy section (b) demonstrate modest plasmacytosis. Plasma cells show kappa light chain restriction (brown) by dual immunohistochemical stains (c).

Symptomatic PCM

Symptomatic PCM, or myelomatosis, is characterized by monoclonal proliferation of plasma cells in the bone marrow and an M-protein production (see Figures 16.5–16.12).

TABLE 16.4 Criteria for asymptomatic myeloma (smoldering myeloma).*

Serum M-protein	≥3 g/dL
Bone marrow clonal plasma cells	≥10%
No related tissue or organ impairment or symptoms	

*From Ref. [51].

TABLE 16.5 Myeloma-related organ or tissue impairment.*

Elevated serum calcium levels	>0.25 mmol/L above the upper limit of normal or >2.75 mmol/L
Renal insufficiency	Creatinine >173 mmol/L
Anemia	Hemoglobin 2 g/dL below the lower limit of normal or <10 g/dL
Bone lesions	Lytic lesions or osteoporosis with compression fractures
Others	Symptomatic hyperviscosity, amyloidosis, recurrent bacterial infections

*From Ref. [51].

TABLE 16.6 Criteria for the diagnosis of plasma cell myeloma set by the International Myeloma Working Group.*

M-protein in serum and/or urine
Bone marrow clonal plasma cells or plasmacytoma**
Related organ or tissue impairment (end-organ damage, including bone lesions)***

*From Ref. [51].

**Immunophenotypic studies demonstrate monoclonal population of abnormal plasma cells.

***Some patients may have no symptoms but show evidence of organ or tissue impairment (see Table 15.3).

This neoplastic proliferation leads to bone destruction and pathological fractures, particularly in the spine and ribs [51]. Anemia, hypercalcemia, and renal insufficiency are other common features (Table 16.5). The criteria for the diagnosis of PCM proposed by the IMWG are shown in Table 16.6. According to the IMWG, the most critical criterion for symptomatic myeloma is the evidence of organ or tissue impairment manifested by anemia, hypercalcemia, lytic bone lesions (Figure 16.22), renal insufficiency, hyperviscosity, amyloidosis, or recurrent infections [51]. Unlike the WHO criteria for the diagnosis of PCM (Table 16.7), no level of serum or urine M-protein and no minimal percentage of clonal bone marrow plasma cells are

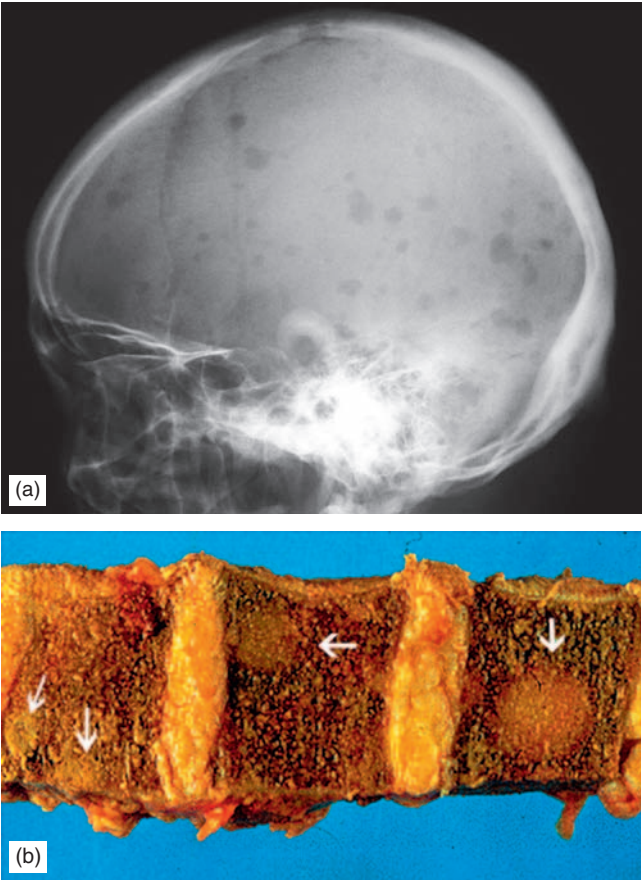


FIGURE 16.22 (a) Skull X-ray of a patient with plasma cell myeloma demonstrating punched out lytic lesions. (b) Gross specimen of a vertebrate showing several bone marrow lesions of plasma cell myeloma (arrows).

TABLE 16.7 WHO diagnostic criteria for plasma cell myeloma.*

<i>Major criteria</i>
A. Marrow plasmacytosis (>30%)
B. Plasmacytoma on biopsy
C. M-component:
i. Serum: IgG > 3 g/dL, IgA > 2 g/dL
ii. Urine > 1 g/24 h of Bence-Jones protein
<i>Minor criteria</i>
A. Marrow plasmacytosis (10–30%)
B. M-component present but less than above
C. Lytic bone lesions
D. Reduced normal Ig (<50% normal levels)
The diagnosis of plasma cell myeloma requires a minimum of one major and one minor criteria or three minor criteria which must include (A) and (B).

*From Ref. [1].

required in the proposed diagnostic criteria by the IMWG. Approximately 5% of patients with evidence of tissue or organ impairment may demonstrate <10% plasma cells in their bone marrow, and therefore based on the IMWG

criteria, they should carry the diagnosis of PCM [51] and be treated appropriately.

Approximately 50% of the patients with PCM show IgG, 25% IgA M-proteins, and about 20% demonstrate only monoclonal light chain production [29, 30, 57]. IgD and IgM PCM are rare [58, 59]. Bence-Jones protein is detected in the urine of about 75% of patients. Over 95% of the patients with PCM show an M-protein in the serum or urine at the time of diagnosis [51]. Approximately 20% of patients show hypercalcemia. Conventional radiologic studies reveal lytic bone lesions, osteoporosis, or fractures in about 80% of the patients at diagnosis.

The bone marrow biopsies more often show focal lesions than diffuse, and therefore repeated biopsies may be required to establish the diagnosis. The morphologic features of the neoplastic plasma cells may vary from normal-appearing mature forms to blastic or anaplastic forms. As mentioned earlier, the neoplastic plasma cells, unlike normal plasma cells, lack the expression of CD19 and may show aberrant expression of CD56, CD10, and a number of other myeloid or T-cell-associated CD molecules [30–34].

An international staging system has been proposed based on the serum beta-2 microglobulin ($\beta 2M$) and serum albumin levels [30, 60]:

Stage I: Serum $\beta 2M$ <3.5 mg/L and serum albumin ≥ 3.5 g/dL

Stage II: Neither stage I nor stage III

Stage III: Serum $\beta 2M \geq 5.5$ mg/L

The median survival times for patients with stages I, II, and III are 62, 44, and 29 months, respectively [30].

Hyperdiploidy and $t(11;14)(q13;q32)$ are reported in association with favorable prognosis (median survival ≥ 50 months), and $t(4;14)(p16;q32.3)$, $t(14;16)(q32.3;q23)$, $del(17p13)$ (locus for $p53$), and hypodiploidy are considered indicators of poor prognosis (median survival 25 months). The $del(13q14)$ is associated with an intermediate prognosis with median survival of 42 months. In one flow cytometric study, patients with >10 circulating plasma cells (CD38+, CD45–) per 50,000 mononuclear cells had a significantly lower median survival than those with ≤ 10 circulating plasma cells [61]. Table 16.8 provides a list of the adverse prognostic factors. Conventional chemotherapy, autologous and allogeneic stem cell transplantation, and more recently targeted therapies have been used to increase the survival rate [29].

Non-secretory Myeloma

Non-secretory myeloma accounts for 1–5% of all myelomas and is characterized by the absence of detectable M-protein in the serum and urine [62, 63]. However, utilization of more sensitive techniques such as serum-free light chain assay may significantly reduce the number of these cases [64, 65]. The reports on non-secretory myelomas suggest a lower incidence of renal failure and hypogammaglobulinemia, lower median percentage of bone marrow plasma cells, higher incidence of neurological presentation, and longer survival than the secretory myelomas [66]. The therapeutic

TABLE 16.8 Adverse prognostic factors in plasma cell myeloma.*

Age	≥70 years
Serum albumin	<3 g/dL
Serum creatinine	≥2 mg/dL
Beta-2 microglobulin	>4 mg/L
Plasma cell labeling index	≥1%
Circulating plasma cells	>10 per 50,000 mononuclear cells
Serum calcium	≥11 mg/dL
Platelet count	<15,000/ μ L
Hemoglobin	<10 g/dL
Cytogenetics	t(4;14)(p16;q32.3) t(14;16)(q32.3;q23) del(17p13) (p53 locus) hypodiploidy

* Adapted from Kyle RA, Gertz MA, Witzig TE, Lust JA, Lacy MQ, Dispenzieri A, Fonseca R, Rajkumar SV, Offord JR, Larson DR, Plevak ME, Therneau TM, Greipp PR. (2003). Review of 1027 patients with newly diagnosed multiple myeloma. *Mayo Clin Proc* **78**, 21–33, and Ref. [30].

approaches for non-secretory myeloma are similar to those for secretory PCM.

Plasma Cell Leukemia

Plasma cell leukemia is a rare event characterized by the presence of $>2,000/\mu\text{L}$ of circulating plasma cells accounting for $>20\%$ of the white cell differential count (Figure 16.23) [1, 67–69]. Plasma cell leukemia is divided into two categories: primary and secondary. Primary plasma cell leukemia constitutes about 60% of the cases and is manifested *de novo* without evidence of previous history of PCM. Secondary plasma cell leukemia accounts for the remaining 40% and represents leukemic transformation in patients with a history of PCM. Patients with primary plasma cell leukemia are younger, have fewer lytic bone lesions and smaller amounts of serum M-protein, demonstrate higher incidence of hepatosplenomegaly, and show a longer survival than patients with secondary plasma cell leukemia [51]. The immunophenotype of primary plasma cell leukemia is frequently IgD, IgE, or light chain only [1], but clonal gene rearrangement studies targeting the IgM region will be sufficient to establish a monoclonal plasma cell disorder if needed.

PLASMACYTOMA

Plasmacytoma is a solitary neoplasm of plasma cells involving bone or extramedullary sites. Plasmacytoma demonstrates identical morphologic and immunophenotypic features of PCM [1].

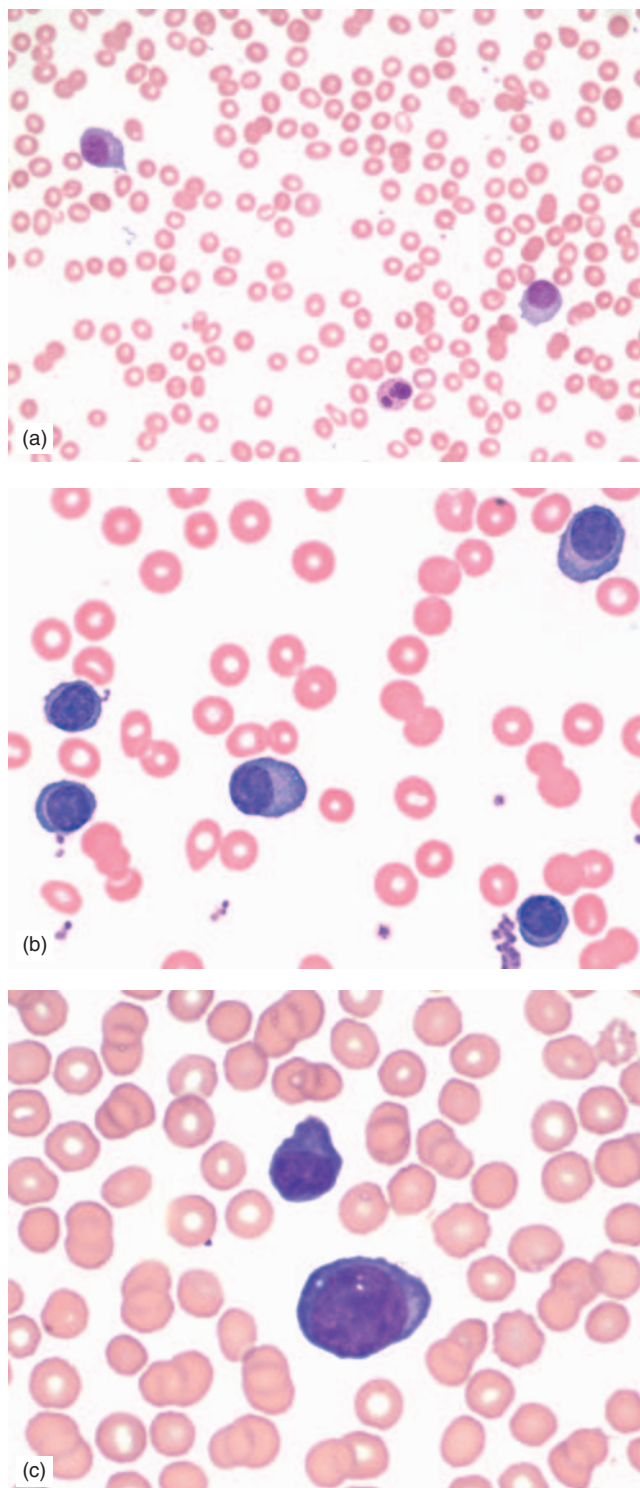


FIGURE 16.23 Blood smears of patients with plasma cell leukemia demonstrating circulating plasma cells with various morphologic features (a, b and c). © From Naeim F. (1997). *Pathology of Bone Marrow*, 2nd ed. Williams & Wilkins, Baltimore, by permission.

Solitary Plasmacytoma of Bone

Solitary plasmacytoma of bone is a rare condition and accounts for about 3–5% of the plasmacytic neoplasms. The

TABLE 16.9 Characteristics of plasmacytoma of bone and extramedullary sites.*

No M-protein in serum or urine**
Solitary bone or extramedullary tumor of monoclonal plasma cells
Bone marrow not consistent with plasma cell myeloma
Normal skeletal survey
No related organ or tissue impairment

*From Ref. [51].

**A small amount of M-component may be present in some cases.

median age is around 55 years and the male:female ratio is about 2:1. It often involves the axial skeleton, particularly thoracic vertebrae and ribs. Involvement of distal bones, particularly below the knees or elbows, is extremely rare [51, 70]. Bone pain at the site of the lesion is one of the most common presenting symptoms. Infiltration of the tumor cells into the surrounding soft tissue may result in a palpable mass. Radiologic studies show no evidence of additional lesions. Morphologic and immunophenotypic features are identical to those of PCM, but no serum or urine M-protein is detected, and there is no evidence of anemia, hypercalcemia, renal insufficiency, or other organ or tissue impairment (Table 16.9). Multiple solitary plasmacytomas without evidence of PCM occur in up to 5% of the cases [51]. Approximately 50% of patients with solitary plasmacytoma of bone eventually develop PCM [51]. Plasmacytomas of >5 cm in diameter have a greater chance of conversion to PCM. Radiotherapy is the treatment of choice.

Extramedullary Plasmacytoma

Extramedullary plasmacytoma is a monoclonal plasma cell neoplasm that arises outside the bone marrow. The most frequent site of involvement is the upper respiratory tract, including the nasal cavity and sinuses, nasopharynx, and larynx [51, 70–74], but any organ or tissue may be involved, such as gastrointestinal tract and urinary tracts, thyroid, male and female reproductive systems, parotid gland, lymph nodes, and central nervous system (Figure 16.24). The diagnosis is made based on the monoclonality of the plasma cell tumor and lack of evidence for PCM and serum or urine M-protein (Table 16.9). IgA is the most frequent immunophenotype, but again it is IgM that is typically assessed at the molecular level for monoclonality (Figure 16.25). Approximately 15% of the patients may eventually develop symptomatic PCM [74]. Surgery and/or radiation are the treatment of choice.

OSTEOSCLEROTIC MYELOMA

Osteosclerotic myeloma, or POEMS syndrome, is characterized by a combination of peripheral neuropathy (P), organomegaly (O), endocrinopathy (E), monoclonal plasma

cell disorder (M), and skin changes (S) [75, 76]. Other frequent features include sclerotic bone lesions, Castleman's disease, papilledema, serous effusions, and thrombocytosis. The biopsy sections of sclerotic lesions show a monoclonal population of plasma cells. Plasmacytosis is usually modest (median 5%), but the bone marrow is often hypercellular with myeloid preponderance, increased megakaryocytes, and thick bone trabeculae. The M-protein is typically small and sometimes undetectable by routine serum protein electrophoresis [76]. The median age and survival reported in a Mayo Clinic study of 99 patients were 51 years and 165 months, respectively [76].

MONOCLONAL IMMUNOGLOBULIN DEPOSITION DISEASES

Monoclonal immunoglobulin deposition diseases are monoclonal gammopathies characterized by the deposition of Ig-derived proteins in the organs and tissues causing impairment of their function. These disorders are divided into two major groups: (1) disorders with the deposition of fibrillary proteins (primary amyloidosis) and (2) disorders with the deposition of an amorphous, non-fibrillary protein, known as monoclonal light and heavy chain deposition diseases.

Primary Amyloidosis

“Amyloidosis” is a general term referring to a heterogeneous group of disorders characterized by the extracellular deposition of fibrillar proteins with antiparallel beta-pleated sheet configuration on X-ray diffraction [77–85]. These fibrillar structures are identified on biopsy sections by an intense yellow-green fluorescence by thioflavine T and by binding to Congo red stain, leading to apple green birefringence under polarized light [77, 78]. So far, 23 different proteins have been identified that form fibrillar extracellular amyloid deposits in tissues that bind Congo red [79]. The major categories of amyloidosis include (1) primary amyloidosis, (2) secondary amyloidosis, (3) dialysis-related amyloidosis, (4) heritable amyloidosis, and (5) senile amyloidosis (Table 16.10).

Primary amyloidosis (AL) is referred to a specific type of amyloidosis in which the fibrillar protein is derived from monoclonal Ig light chains. Primary amyloidosis is considered a variant of monoclonal plasma cell proliferative disorder, and in about 10% of the cases it is associated with symptomatic PCM.

In approximately 75% of the cases, the fibrillar protein is derived from the variable region of lambda light chain. The kappa light chain is involved in the remaining 25%. There appears to be a correlation between the site of involvement and the involved variable region of the light chain. For example, the amyloid deposit in patients with dominant renal involvement is often derived from V lambda IV, whereas in patients with dominant cardiac involvement the amyloid deposit is derived from V lambda II or III [77–85]. A serum or urinary monoclonal protein could be

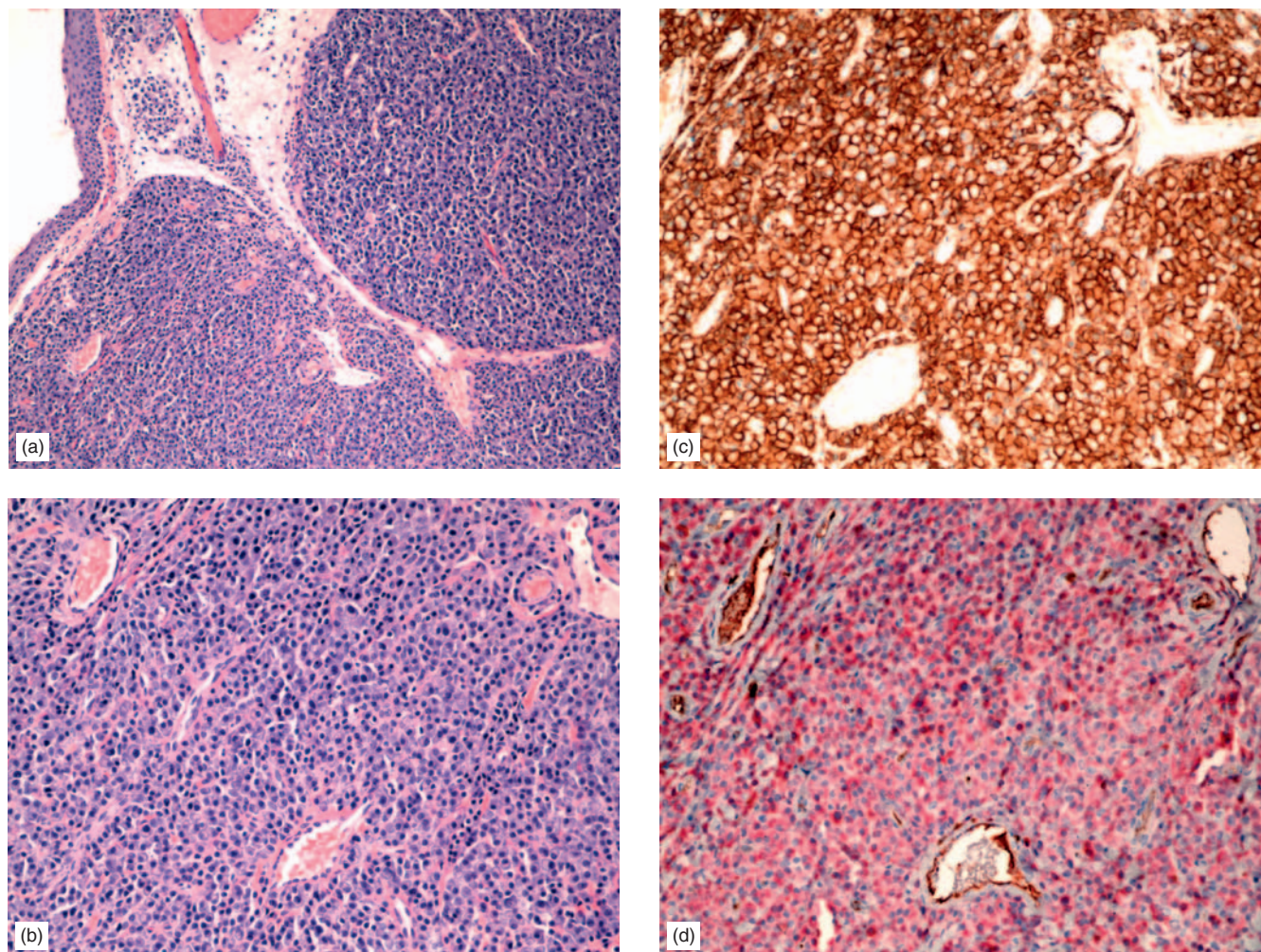


FIGURE 16.24 Solitary plasmacytoma of conjunctiva. Biopsy section demonstrating a large aggregate of plasma cells: (a) low power and (b) intermediate power. Immunohistochemical stains for CD138 (c) and lambda light chain (d, pink) demonstrate a monoclonal population of plasma cells. Courtesy of G. Pezeshkpour, M.D., Department of Pathology, VA Greater Los Angeles Healthcare System.

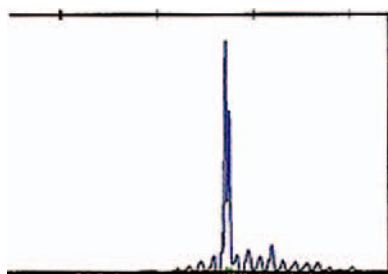


FIGURE 16.25 IgH PCR study of an extramedullary plasmacytoma showing a prominent clonal peak (framework 1 primer set) amid a weaker polyclonal background.

detected in over 85% of the patients using immunofixation techniques. Also, a protein known as serum amyloid P component (SAP) is detected by scintigraphy in patients with primary amyloidosis.

The diagnosis of amyloidosis is based on the biopsies obtained from the affected organs. Liver and kidney biopsies are positive in over 90% of the cases, followed by

abdominal fat pad aspirate and biopsies of rectum, bone marrow (Figure 16.26), and skin [78].

Monosomy of chromosome 18 is the most common abnormality in primary amyloidosis followed by $t(11;14)(q13;q32)$ and $del(13q14)$ [85, 86].

The most frequent clinical symptoms in primary amyloidosis are (1) nephrotic syndrome with or without renal insufficiency, (2) cardiomyopathy, (3) peripheral neuropathy, (4) hepatomegaly, and (5) macroglossia [78]. Elevated serum β -2 microglobulin and bone marrow plasma cells $>10\%$, dominant cardiac involvement, and circulating plasma cells $>1\%$ correlate with poor prognosis [78–83]. The actuarial survival for 810 patients studied at the Mayo Clinic was 51% at 1 year, 16% at 5 years, and 4.7% at 10 years [85]. Progression to PCM is rare and in one large study it was reported in only 0.4% of the patients between 10 and 81 months [86]. Therapeutic approaches include chemotherapy, such as melphalan with or without prednisone or dexamethasone and stem cell transplantation.

The most common form of heritable amyloidosis is familial Mediterranean fever, an autosomal recessive autoinflammatory disorder characterized by periodic

TABLE 16.10 Major categories of amyloidosis.*

Amyloid protein	Precursor	Clinical status
AL	Ig light chain	Primary amyloidosis, local or systemic, associated with monoclonal plasma cell disorders
AA	Apolipoprotein AA	Secondary amyloidosis, systemic, associated with chronic infections
A β 2M	Beta-2 microglobulin	Hemodialysis, systemic
AApoAI, AApoAII, AGel, ALys, ACys, and others	Apolipoprotein AI and AII, gelsolin lysozyme, cystatin C, and others	Familial, systemic
Ab, APro, ATTR, AMed	Ab protein precursor, prolactin, transthyretin, lactadherin	Senile, local, or systemic

*Adapted from Ref. [79].

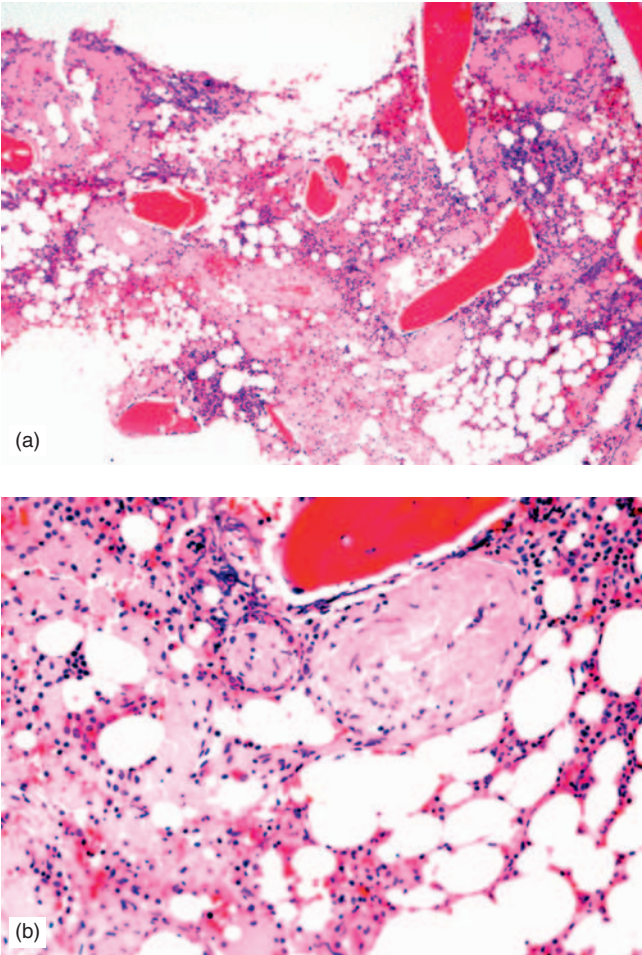


FIGURE 16.26 Bone marrow biopsy section demonstrating primary amyloidosis: (a) low power and (b) high power views.

fevers, abdominal pain (peritonitis), pleuritis, arthritis, pericarditis, and skin rash. It is most prevalent in individuals of Armenian descent, in whom the carrier frequency approaches 1 in 7 but is also found in Arabs, Jews, Persians, Italians, and Greeks [1, 87–90]. In addition to the periodic attacks of pain and fever, the life-threatening complication of the disease is amyloid deposition, particularly in the kidneys. However, this form of amyloidosis is to be distinguished from the others discussed here since it is neither related to plasma cell proliferation nor is it composed predominantly of Ig protein components. Differential diagnosis is made by the clinical history and genetic testing. The causative gene, *MEFV*, will often, but not always, demonstrate mutations in the homozygous or compound heterozygous state. Since over 60 different mutations have been reported, practical testing is usually limited to a subset of the more common ones found in Mediterranean populations [91]. Technical approaches include DNA sequencing (Figure 16.27a) and allele-specific DNA probe hybridization (Figure 16.27b).

Monoclonal Light and Heavy Chain Diseases

The light chain deposition disease (LCDD) and heavy chain deposition disease (HCDD) are clinical variants of monoclonal plasma cell disorders characterized by the deposition of abnormal light chain, heavy chain, or both in the tissues or organs [75, 92, 93]. The deposits, unlike primary amyloidosis, are not fibrillar, do not bind Congo red, and do not contain SAP. LCDD is more common than primary amyloidosis and often consists of kappa light chain (Table 16.11). The primary defect in LCDD appears to be mutations in the Ig light chain variable region, with predominant involvement of V_{KIV} of kappa light chain [1, 78, 29].

Deletion of the *CH1* constant domain and point mutation of variable regions of the heavy chain are the primary events in HCDD [1, 78, 93]. These events lead to premature secretion of heavy chain binding protein and increased tendency for tissue deposition. HCDD of IgG1 and IgG3 isotypes are associated with reduced complement activities [1, 94]. Many organs may be involved, including kidneys, liver, heart, nerves, and blood vessels. Approximately 85% of the cases show a detectable serum M-component.

Heavy Chain Diseases

The heavy chain disease is a monoclonal lymphoplasmacytic disorder characterized by the production of incomplete Ig molecules because of the lack of gamma chain binding sites for light chains. There are three major categories of heavy chain diseases: α , γ , and μ [1, 95, 96].

The α heavy chain disease (α HCD, Mediterranean lymphoma) is considered a variant of MALT-type lymphoma [1]. It occurs in older children and young adults and is associated with gastrointestinal symptoms, such as malabsorption, intestinal obstruction, and diarrhea [1, 97–99].

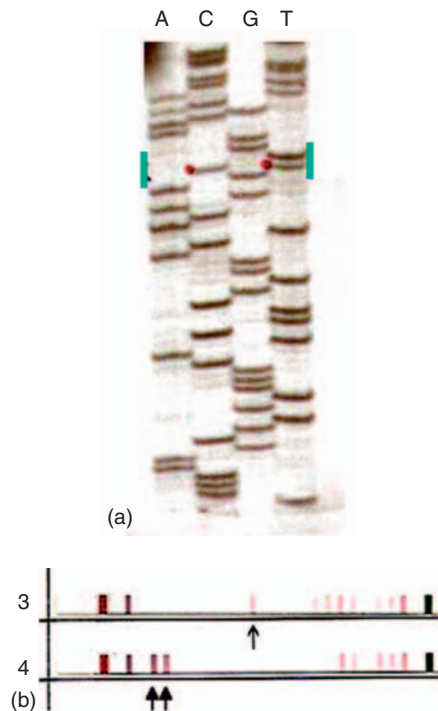


FIGURE 16.27 (a) DNA sequencing gel showing heterozygosity for the V726A mutation in the 2MEFV gene of a patient with familial Mediterranean fever (the second mutation was M694V). The red dots mark the nucleotide position showing both T (normal) and C (mutant). (b) Reverse hybridization strips using allele-specific oligonucleotide probes directed against the most prevalent mutations in the MEFV gene associated with familial Mediterranean fever. Patient #3 is homozygous for mutation K695R (single arrow), and patient #4 is compound heterozygous for mutations P369S and E148Q (double arrows).

TABLE 16.11 Comparison between primary amyloidosis and light chain deposition disease.

Type	Ig	Deposition	Congo red
Primary amyloidosis	Light chain, often λ	Fibrillary	Positive
Light chain deposition	Light chain, often κ	Non-fibrillary	Negative

α HCD is the most frequent heavy chain disease with the majority of the reported cases being from Middle East, North and South Africa, and the Far East. The pathologic features are more or less similar to those described in MALT lymphoma depicted by a mucosal infiltrate of centrocyte-like lymphocytes and plasma cells. An abnormal heavy chain protein is detected in the serum of 20–90% of the patients [1, 97]. Antibacterial therapy may completely resolve the disease in early stages. Some cases may eventually transform to large B-cell lymphoma. Despite the similar ethnic appellation, this disorder has nothing to do with familial Mediterranean fever, which is not a B-cell disorder.

The γ heavy chain disease (γ HCD, Franklin disease) is a rare condition presenting with lymphadenopathy, splenomegaly, and hepatomegaly with lymphoplasmacytic infiltration similar to lymphoplasmacytic lymphoma [1, 100]. Immunofixation studies may show the presence of serum IgG without light chain. Some patients may demonstrate autoimmune disorders or chronic lymphocytic leukemia [100].

The μ heavy chain disease is a rare lymphoproliferative disorder with clonal IgM molecules with defective variable region [101–103]. Clinically, it resembles chronic lymphocytic leukemia and is often associated with hepatosplenomegaly [1]. The bone marrow is infiltrated by mature small lymphocytes admixed with vacuolated plasma cells [1]. Lymphadenopathy is unusual.

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Mature T-Cell and NK-Cell Neoplasms

Mature T- and NK-cell neoplasms represent a wide spectrum of lymphoid malignancies developed from the clonal proliferation of mature T- and NK-cells. These disorders may involve bone marrow and peripheral blood (leukemia), lymphoid or extramedullary tissues (lymphoma), or both. Mature T- and NK-cell neoplasms comprise <15% of all lymphoid tumors. According to the World Health Organization (WHO) classification, they are divided into three major categories: (1) leukemic or disseminated, (2) nodal, and (3) extranodal [1] (Table 17.1).

TABLE 17.1 WHO classification of mature T- and NK-cell neoplasms.*

Leukemic or disseminated

T-cell prolymphocytic leukemia
T-cell large granular lymphocytic leukemia
Aggressive NK-cell leukemia
Adult T-cell leukemia/lymphoma

Nodal

Peripheral T-cell lymphoma, unspecified
Angioimmunoblastic T-cell lymphoma
Anaplastic large cell lymphoma

Extranodal

Cutaneous
Blastic NK-cell lymphoma**
Mycosis fungoides/Sézary syndrome
Primary cutaneous anaplastic large cell lymphoma
Other extranodal
Extranodal NK/T-cell lymphoma, nasal type
Enteropathy-type T-cell lymphoma
Hepatosplenic T-cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma

*Adapted from Ref. [1].

**Known also as agranular hematodermic neoplasm it is apparently derived from plasmacytoid dendritic cells and not from NK-cells.

T-CELL PROLYMPHOCYTIC LEUKEMIA

T-cell prolymphocytic leukemia (T-PLL) is a sporadic and aggressive lymphoproliferative disorder of post-thymic T-cells characterized by a high peripheral blood lymphocyte count and infiltration of the bone marrow, spleen, liver, lymph nodes, and skin [1–3].

Etiology and Pathogenesis

The etiology and pathogenesis of T-PLL are not known. A high frequency of ataxia-telangiectasia (*ATM*) gene mutations suggests that *ATM* functions as a type of tumor suppressor gene [4–6]. Most T-PLL cases also show an aberrant T-cell receptor alpha (*TCRA*) gene rearrangement that activates *TCL1* or *MTCP1-B1* oncogenes [4].

Pathology

Morphology

T-PLL refers to a group of mature T-cell leukemias with a diverse morphology but similar clinical outcome. The observed morphologic variations include [1]:

1. Cells with typical prolymphocytic features: medium-sized lymphocytes with variable amount of non-granular basophilic cytoplasm; round, oval, or irregular nucleus; coarse chromatin; and a single prominent nucleolus (Figures 17.1 and 17.2). T-prolymphocytes often show cytoplasmic blebs. The prolymphocytic morphology accounts for about 70% of the cases.

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2. Small lymphocytes often with irregular nuclei and indistinct nucleolus [7]. This morphologic subtype was previously referred to as T-CLL, but now it is included in T-PLL, because of similar biological behavior. It represents about 25% of the cases [1].
3. Approximately 5% of the cases may show lymphoid cells with cerebriform (Sezary-like) nuclei [1, 8, 9].

The peripheral blood and bone marrow are the primary sites of involvement. There is marked peripheral blood lymphocytosis, usually $>100,000/\mu\text{L}$, often with anemia and thrombocytopenia. The bone marrow is commonly infiltrated in a diffuse or nodular pattern. Splenic infiltration consists of the involvement of both white and red pulps. Skin is affected in about 20% of the cases with dense infiltration of the dermis without epidermal infiltration. The involved lymph nodes are diffusely infiltrated, primarily in the paracortical areas. The remnants of follicular structures may be present [1].

Immunophenotype

The neoplastic cells in the majority of T-PLL cases are of helper T phenotype, expressing CD4 and pan-T-cell markers CD2, CD3, CD5, and CD7. They lack the expression of CD1a and TdT. In approximately 15% of the cases neoplastic prolymphocytes are CD8+, and in about 25% of the cases they coexpress CD4 and CD8 (Figure 17.3) [1, 10].

Cytogenetic and Molecular Studies

Analogous to the immunoglobulin (IgH) receptor loci that are frequently affected by translocations in B-cell lymphomas, the T-cell receptor (TCR) gene loci are targeted by chromosomal breakpoints in approximately 30% of precursor T-cell lymphoblastic leukemias/lymphomas involving various translocation partners. Conventional cytogenetic studies have shown the presence of complex karyotypes and some recurrent chromosomal abnormalities: the most frequent being $t(14;14)(q11.2;q32)$ (Figure 17.4), $inv(14)(q11.2q32)$ (Figure 17.5), $t(X;14)(q28;q11.2)$, $i(8)(q10)$, and $t(8;8)(p12;q11.2)$ [11].

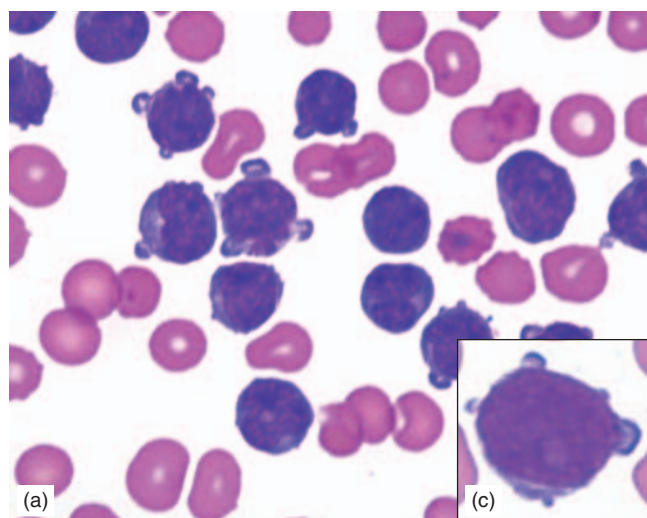


FIGURE 17.1 T-prolymphocytic leukemia. Blood (a) and bone marrow (b) smears demonstrating numerous prolymphocytes with cytoplasmic blebs (c) inset of (a).

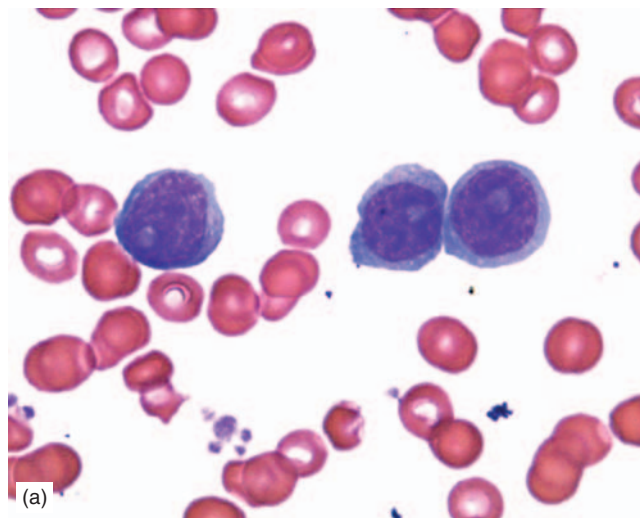


FIGURE 17.2 T-prolymphocytic leukemia. Blood (a) and bone marrow (b) smears demonstrating numerous prolymphocytes.

The $\text{inv}(14)(\text{q}11.2\text{q}32)$ or $\text{t}(14;14)(\text{q}11.2;\text{q}32)$ is reported in over 70% of the cases. These translocations juxtapose *TCRαδ* (14q11.2) and *TCL1* (14q32) genes. The abnormalities of chromosomes 8, 11, 14, and X in T-prolymphocytic leukemia

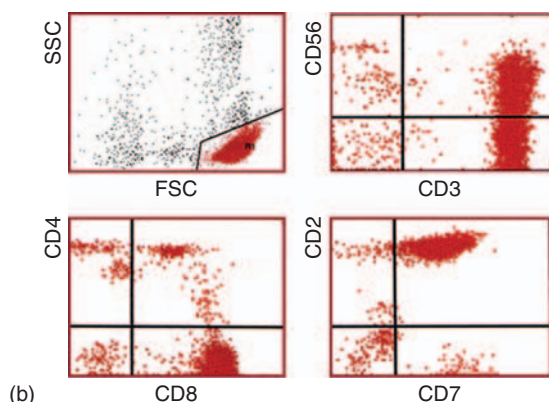
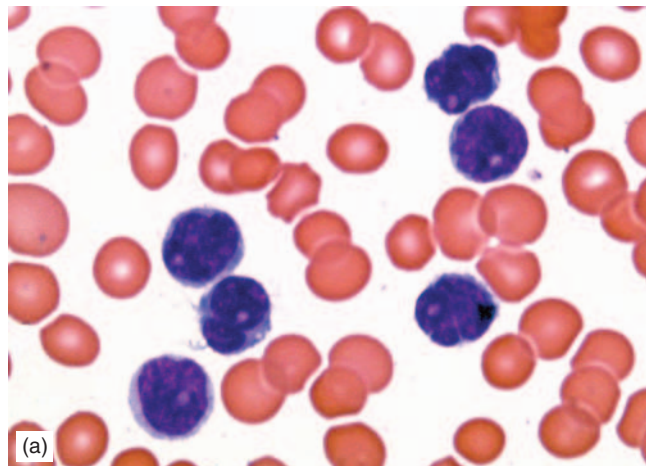


FIGURE 17.3 Flow cytometric analysis of a case of T-prolymphocytic leukemia demonstrating a less common cytotoxic phenotype. The neoplastic cells express CD2, CD3, CD5, CD8, and partial CD56.

have been identified by fluorescence *in situ* hybridization (FISH) studies. Other more consistent aberrations include the loss of 8p, 11q, 22q11, 13q, 6q, 9p, 12p, 11p11-p14, and 17p. In addition, four regions of gain at 8q, 14q32, 22q21-qter, and 6p are also observed. The Xq28 (*MTCP1* gene locus) or the 14q32.1 regions are involved in translocations or inversions with the *TCRαδ* at 14q11.2. Translocations involving the *TCR* loci either *TCRαδ* at 14q11.2 or *TCRβ* at 7q35 have been detected in 15–33% of the patients [12]. These translocations lead to a deregulated expression of the partner gene by juxtaposition with the regulatory region of one of the *TCR* loci. The *TCR* breakpoints in many cases resemble *TCR* recombination signals, implying that the genetic alteration occurred during *TCR* rearrangement. The inactivation of the *ATM* gene by deletion and mutation is consistently found in T-PLL. *ATM* mutations have been detected in over 50% of the T-PLL cases suggesting that *ATM* acts as a tumor suppressor gene [4].

Recent studies using FISH for loss of heterozygosity (LOH) and comparative genomic hybridization (CGH) analyses have identified losses of the 11q21-q23 region in most of the T-PLL cases. The *ATM* gene located in this region has been shown to be lost by these deletions (Figure 17.7). Other chromosomal aberrations include $\text{der}(11)\text{t}(1;11)(\text{q}21;\text{q}23)$ (Figure 17.6); $\text{t}(X;7)(\text{q}28;\text{q}35)$, $\text{t}(X;14)(\text{q}28;\text{q}11)$, and $\text{t}(3;22)(\text{q}21;\text{q}11.2)$ have also been reported [5, 11].

At the molecular level, the rearrangements of *TCR* and the involvement of *TCL1*, *MTCP1-B1*, and *ATM* genes are common findings. *ATM* mutations have been detected in over 50% of the T-PLL cases suggesting that *ATM* acts as a tumor suppressor gene [3–5]. Unfortunately, the *ATM* gene is extremely large, and detection of mutations, which requires extensive gene sequencing, is not routinely available for this purpose.

TCR gene rearrangements are detected in the same general manner as for immunoglobulin gene rearrangements in B-cell malignancies (see Chapter 15). However, they are often of more crucial importance to the case, since one does not have the advantage of surface immunoglobulin

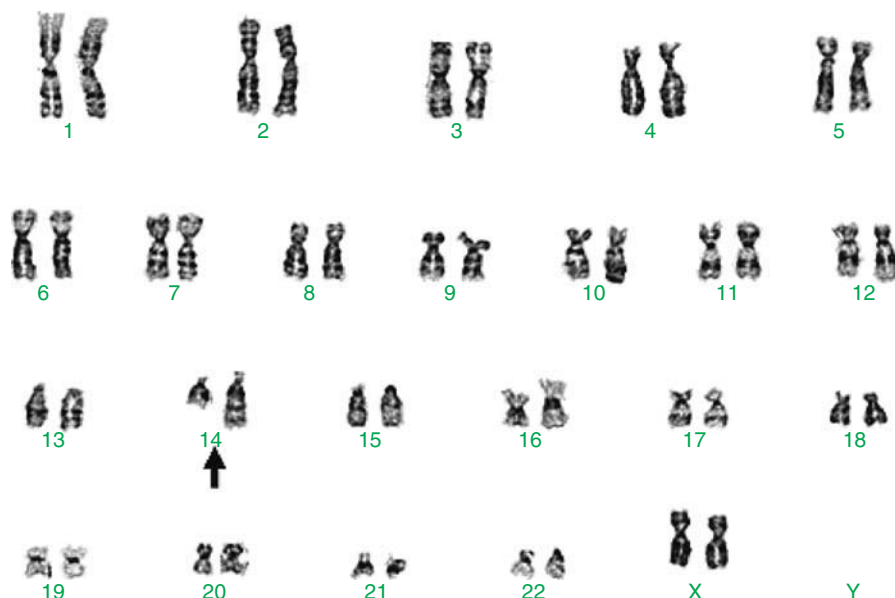


FIGURE 17.4 Karyotype of tumor cells in a patient with T-prolymphocytic leukemia demonstrating $46,\text{XX},\text{t}(14;14)(\text{q}11.2;\text{q}32)$.

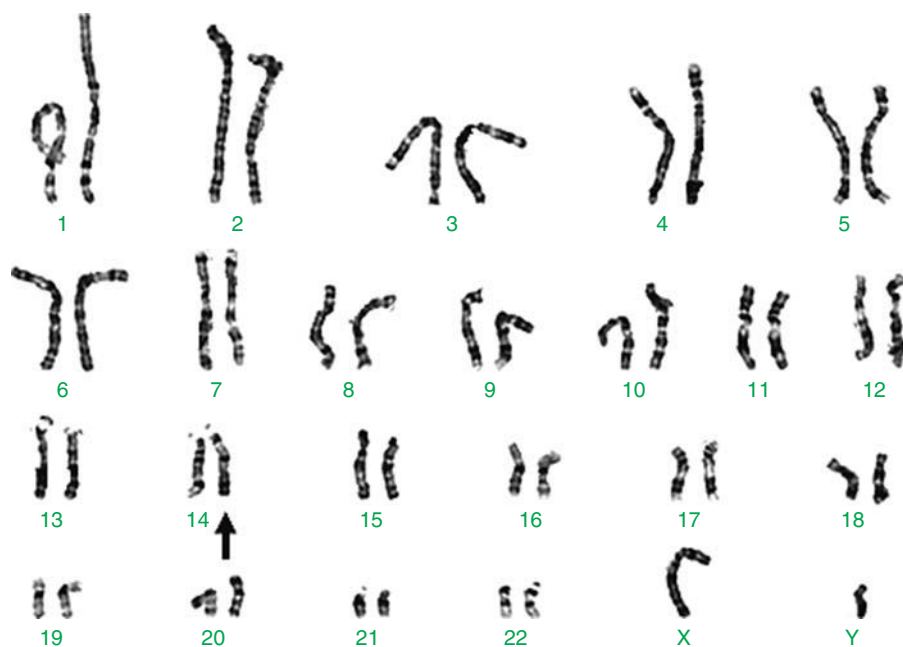


FIGURE 17.5 Karyotype of tumor cells in a patient with T-prolymphocytic leukemia demonstrating 46,XY,inv(14)(q11.2;q32).

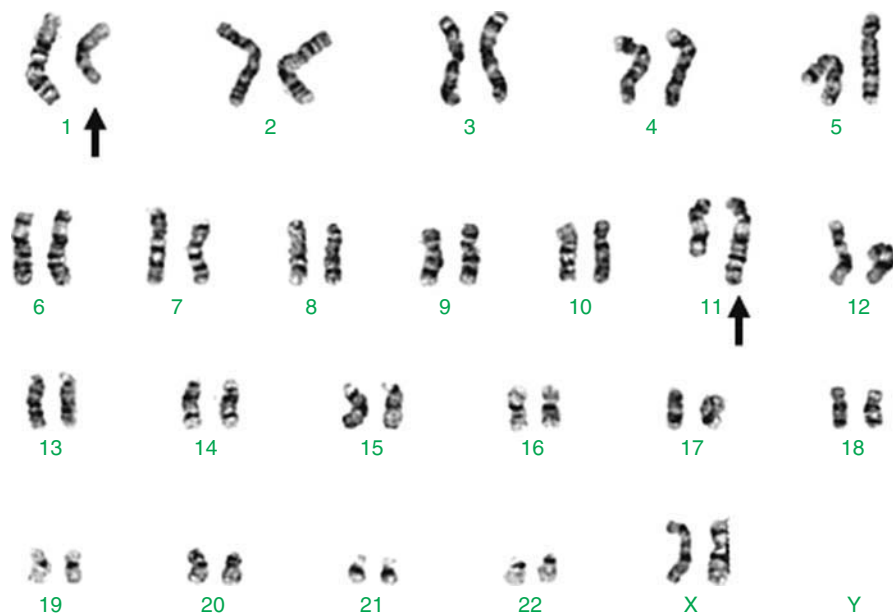


FIGURE 17.6 Karyotype of tumor cells in a patient with T-prolymphocytic leukemia demonstrating 46,XX,t(1;11)(q21;q23).

immunophenotyping (light-chain restriction) as ancillary evidence of clonality. Again, the Southern blot method will pick up a greater proportion of clonal rearrangements because it is capable of surveying a larger span of the target gene region. Most laboratories use a probe directed to the constant region of the beta-chain genes ($TC\beta$) on chromosome 7q34. This is because the most circulating T-cells are of the $\alpha\beta$ type. But as laboratories have moved away from the cumbersome Southern blot procedure, various polymerase chain reaction (PCR) strategies have been developed. Unlike the J_H region targeted in B-cell lesions, however, the $TCR\beta$ region is very large and complex, and to cover it adequately (i.e. with pick-up rate approaching that of Southern blot) requires the use of a large number of primers

[13] which can itself be quite cumbersome and difficult to interpret. However, the TCR genes do not undergo somatic hypermutation as do the immunoglobulin genes, eliminating an important cause of false-negative PCR results seen in the B-cell lesions. One compromise is to target the $TCR\gamma$ locus, which has far fewer V and J genes, and thus a less complex array of rearrangements must be detected. Moreover, because the γ genes typically rearrange before the β genes, this approach should be equally sensitive as $TCR\beta$ PCR testing [14]. However, the relatively more limited number of $TCR\gamma$ rearrangements can produce a type of false-positive result known as *pseudoclone*; if the total number of T-cells in the submitted specimen is scant, preferential amplification of a small number of these cells that happen to have the same

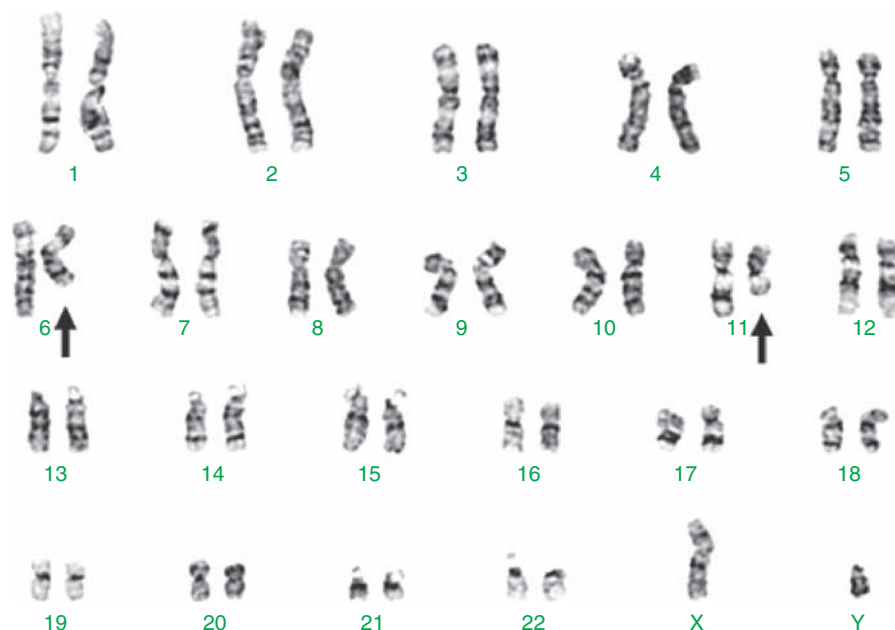


FIGURE 17.7 Karyotype of tumor cells in a patient with T-prolymphocytic leukemia demonstrating 46,XY,del(6)(q13q23),del(11)(q21).

rearrangement pattern can give the appearance of a clone when there is really none. This is unlikely to occur in a leukemia specimen, but may be seen in tissue samples such as skin biopsies.

Clinical Aspects

T-PLL is a rare sporadic T-cell lymphoproliferative disorder, often presented with hepatosplenomegaly and generalized lymphadenopathy [1, 3, 15]. Most of the patients are older than 50 years. The peripheral lymphocyte count is markedly elevated (usually $>100,000/\mu\text{L}$), commonly associated with anemia and thrombocytopenia. Skin infiltration and serous effusions may be observed. The disease typically has an aggressive course with a median survival of <1 year, though occasional cases with spontaneous remission have been reported [16]. An indolent form of T-PLL has also been reported with $t(3;2)(q21;q11.2)$ and elevated serum $\beta 2$ -microglobulin [4]. Combination chemotherapy, and more recently treatment with monoclonal anti-CD52 antibodies (CAMPATH-1H), and allogeneic stem cell transplantation have been used with some responses [3, 17, 18].

Differential Diagnosis

The differential diagnosis includes all leukemic lymphoproliferative disorders that have prolymphocytic morphology or cerebriform nuclei. B-prolymphocytic leukemia and prolymphocytic variant of mantle cell lymphoma are of B-cell lineage with their own characteristic immunophenotypic features. The neoplastic cells of Sezary syndrome (SS) often lack CD7 expression, whereas T-PLL cells are typically CD7+. The tumor cells of adult T-cell leukemia/lymphoma (ATL) may mimic the cerebriform variant of T-PLL, but unlike the T-PLL cells, they are positive for human T-lymphotropic virus type I (HTLV-I).

LARGE GRANULAR LYMPHOCYTIC LEUKEMIAS AND RELATED DISORDERS

The large granular lymphocytes (LGLs) account for 8–15% of the peripheral blood lymphocytes (200–400/mL) characterized by abundant cytoplasm with azurophilic granules (Figure 17.8) [19]. The azurophilic granules contain cytolytic components such as perforin and granzymes. Perforin is a cytolytic protein that induces apoptosis by creating pores in the plasma membrane of the target cell. Granzymes are proteases that induce apoptosis in virus-infected cells. The LGLs are divided into two major categories: cytotoxic T- and NK-cells. The LGL T-cells typically express CD3, CD8, and CD57 and show *TCR* gene rearrangement; whereas the NK-cells express CD56, are negative for surface CD3, may express CD8, and do not show *TCR* gene rearrangement (Figure 17.9) [19–21].

Reactive (non-clonal) large granular lymphocytosis is a relatively frequent phenomenon and has been observed in various conditions, such as viral infections, collagen vascular disorders, myelodysplastic syndromes (MDS), non-Hodgkin lymphomas, hemophagocytic syndrome, and in patients with solid tumors [21–24].

The leukemic (clonal) LGL disorders are of either T- or NK-cell type and are characterized by persistent (≥ 6 months) large granular lymphocytosis and often evidence of infiltration of various organs such as bone marrow, spleen, and liver [1, 20, 21]. There is also an extranodal NK/T-cell lymphoma. According to the WHO, these disorders are classified as [1, 25]:

1. T-cell large granular lymphocytic (T-LGL) leukemia
2. Aggressive NK-cell leukemia
3. Extranodal NK/T-cell lymphoma, nasal type.

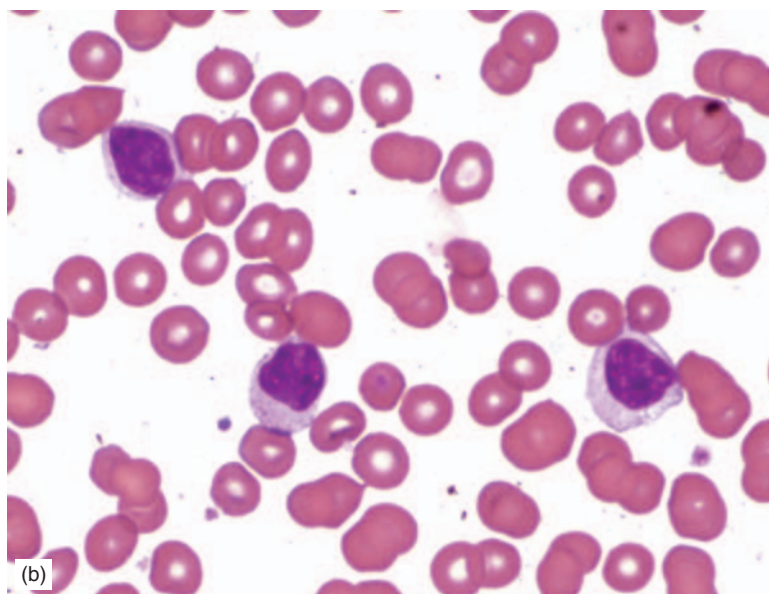
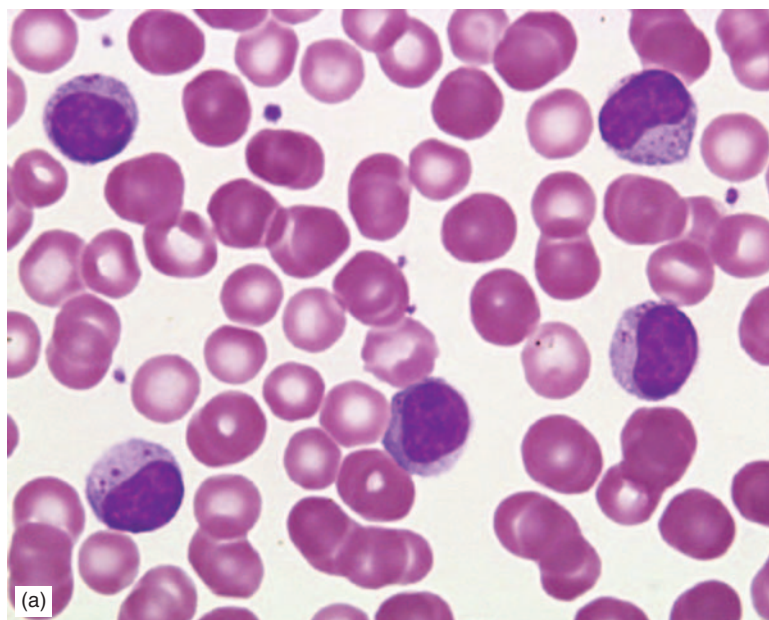


FIGURE 17.8 Large granular lymphocytic (LGL) leukemia. Cytoplasmic granules in LGL cells are usually clearly visible (a), but sometimes difficult to detect (b).

T-CELL LARGE GRANULAR LYMPHOCYTIC LEUKEMIA

The T-cell large granular lymphocytic (T-LGL) leukemia is a chronic lymphoproliferative disorder characterized by persistent T-cell large granular lymphocytosis (usually $>2,000/\mu\text{L}$), cytopenia, and strong association with rheumatoid arthritis [19–21, 25, 26].

Etiology and Pathogenesis

The etiology and pathogenesis of T-LGL leukemia are not known. The strong association of T-LGL leukemia with rheumatoid arthritis suggests that an antigen-driven

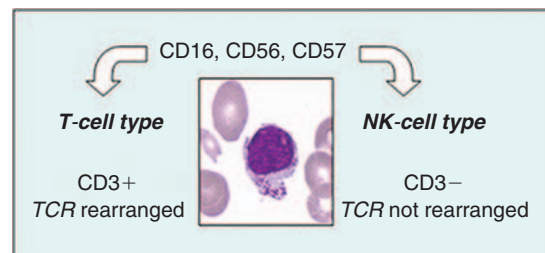


FIGURE 17.9 The major differences between T- and NK-cell types of LGLs.

mechanism may play a role in the initial steps of LGL expansion. Recent studies imply that certain cytokines, such as IL-12 and IL-15, induce proliferation and activation of LGL and, therefore, may play a role in LGL leukemogenesis

[27]. Also, the leukemic LGL cells are able to activate the PI3K–Akt pathway. This pathway regulates the balance between cell survival and apoptosis and has the capacity to block Fas-induced apoptosis [28].

Sera from approximately 50% of the patients with T-LGL leukemia react with HTLV-I/II envelope protein p21e (epitope BA21) in spite of the infrequent detection of the HTLV-II in these patients. These findings suggest the presence of a cellular or retroviral serum protein with homology to the BA21 epitope with a potential role in the LGL leukemogenesis [29, 30].

Pathology

Morphology

The T-LGL leukemia typically shows a persistent (>6 months) absolute large granular lymphocytosis of >2,000/ μ L, often associated with neutropenia or pancytopenia. The total lymphocyte count in most patients is modestly elevated (5,000–10,000/ μ L), but in about one-fourth of the cases it is within normal limits. In a minority of the patients (about 5%), the absolute LGL count is <1,000/ μ L, or the large lymphocytes lack cytoplasmic azurophilic granules, despite their CD3 and CD57 coexpression (see Figure 17.8) [1, 19–22, 25].

Granulocytopenia is observed in over 80% of the patients with approximately half of the patients demonstrating <500/ μ L absolute neutrophil counts [19, 31, 32]. Neutropenia is attributed to different possible mechanisms such as induction of apoptosis in neutrophils by Fas ligand secreted by the leukemic LGL cells, bone marrow infiltration, splenomegaly, or an autoimmune process [28, 32].

Anemia is observed in about 50% of the patients, which may be severe and transfusion dependent (about 20%). The possible mechanisms of anemia include an autoimmune process, splenomegaly, bone marrow infiltration, or pure red cell aplasia. T-LGL leukemia is reported as the most common underlying cause of the pure red cell aplasia. The inhibition of erythroid colony-forming units (CFU-E) and burst-forming units (BFU-E) has been observed by the LGL leukemic cells in patients with pure red cell aplasia [33].

Moderate thrombocytopenia is a frequent finding, due to an autoimmune process, bone marrow infiltration, or secondary to splenomegaly.

The bone marrow is involved in about 90% of the cases. The pattern of leukemic infiltration is usually interstitial and/or sinusoidal. The involvement of the spleen is a common feature with infiltration of the red pulp, often associated with white pulp hyperplasia secondary to the presence of an autoimmune condition (Felty's syndrome). The liver infiltration may involve portal and sinusoidal areas. The involvement of the lymph nodes and other organs is unusual.

Immunophenotype

The T-LGL leukemia cells demonstrate a mature post-thymic phenotype and typically express CD3 (surface and cytoplasmic), CD8, TCR $\alpha\beta$, CD16, CD57, CD122 (IL-2 receptor- β), and TIA-1 (Figure 17.10) [19–21]. There are

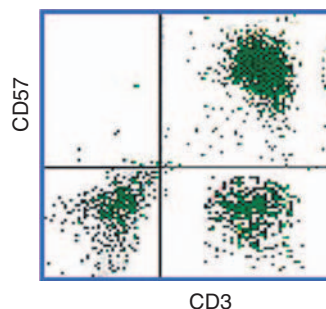


FIGURE 17.10 Flow cytometry of T-LGL leukemia showing coexpression of CD3 and CD57 by tumor cells.

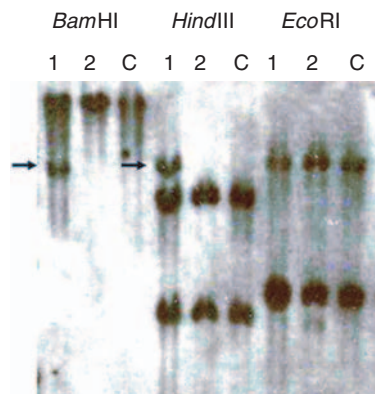


FIGURE 17.11 Clonal *TCRβ* gene rearrangement in a T-LGL leukemia detected by Southern blotting using the *Tcβ* probe. Non-germline, rearranged hybridization bands are seen for patient 1 with two of the three restriction enzymes used (*Bam*HI, *Hind*III, arrows), which is sufficient to diagnose T-cell clonality. Patient 2 shows only germline bands.

minor variants which are characterized by the expression of CD4, CD26, CD56, or TCR $\gamma\delta$. The CD56+ subtype is often associated with an aggressive clinical course [26].

Several monoclonal antibodies are raised against the TCR variable domain and are available for the immunophenotypic analysis of TCR. Of these, monoclonal antibodies against the V β 13.1 region have been reported to be highly associated with T-LGL leukemia [34].

Molecular and Cytogenetic Studies

The T-LGL leukemia cells show *TCRβ* and/or *TCRγ* gene rearrangement by Southern blot or PCR. As noted earlier, *TCRβ* probes are used for Southern blot analysis (Figure 17.11), whereas the *TCRγ* region is most often used as the primary PCR target (Figure 17.12) because it is much smaller and thus easier to amplify with just a few primer sets than is *TCRβ*. In one study, DNA microarray analysis of T-LGL leukemias showed evidence of *IL-1β* gene activation, which was associated with the elevated serum levels of IL-1 β in the majority of the patients [35, 36].

Various cytogenetic abnormalities have been reported in a minority of the T-LGL leukemia cases. These include *inv*(7)(p15q35) (Figure 17.13), *inv*(14)(q11.2q32), *del*(14)(q22-q24) (Figure 17.14), *t*(11;15)(q13;q22-24), *inv*(4)(p14q12), trisomy 8, and trisomy 14 [37, 38].

Clinical Aspects

The T-LGL leukemia accounts for about 85% of all large granular leukemias. The remaining 15% represent the NK type. The median age is around 60 years with only 10% of patients younger than 40 years [19, 39–41]. The clinical symptoms are primarily related to the patient's granulocytopenia and anemia, such as recurrent infections, fever, night sweats, and fatigue. Approximately 30% of the patients are asymptomatic at the time of diagnosis [42]. T-LGL leukemia has been frequently observed in association with connective tissue diseases, primarily rheumatoid arthritis [43–45].

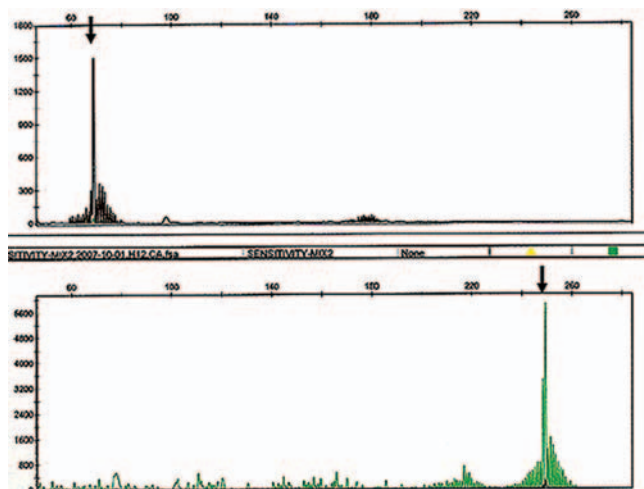


FIGURE 17.12 Clonal *TCRγ* gene rearrangement in a T-LGL leukemia. Discrete clonal PCR peaks are demonstrated above the polyclonal background smear in two of the four *TCRγ* gene regions targeted (Group 1 and AltV γ , arrows).

Rheumatoid factor, antinuclear antibodies, and circulating immune complexes are detected in 40–60% of the patients [22]. Rheumatoid arthritis has been reported in about 25% of the patients with T-LGL leukemia. Many of these patients present the triad combinations of neutropenia, rheumatoid arthritis, and splenomegaly (Felty's syndrome) [46]. Other associated disorders include B-cell lymphoid malignancies, non-Hodgkin lymphoma, thymoma, monoclonal gammopathy, and MDS [19].

The T-LGL leukemia is considered a chronic indolent disorder with reported median survival of >10 years [45]. However, patients with severe neutropenia, "B" symptoms, CD3+, and CD56+ have less favorable prognosis and require treatment [26]. Therapeutic approaches include low-dose chemotherapy with methotrexate, cyclophosphamide, or cytosporin as single agents or in combination with prednisone [19].

AGGRESSIVE NK-CELL LEUKEMIA

Aggressive NK-cell leukemia is characterized by systemic proliferation of LGLs of NK type (NK-LGL), strong Epstein-Barr virus (EBV) association, and an aggressive clinical course [1, 19, 47–49]. A rare indolent EBV-negative condition known as *chronic NK-cell lymphoproliferative disorder* has been described [26]. The neoplastic nature of this indolent condition is uncertain.

Etiology and Pathogenesis

The etiology of the aggressive NK-cell leukemia is not known. The strong association of this disorder with EBV

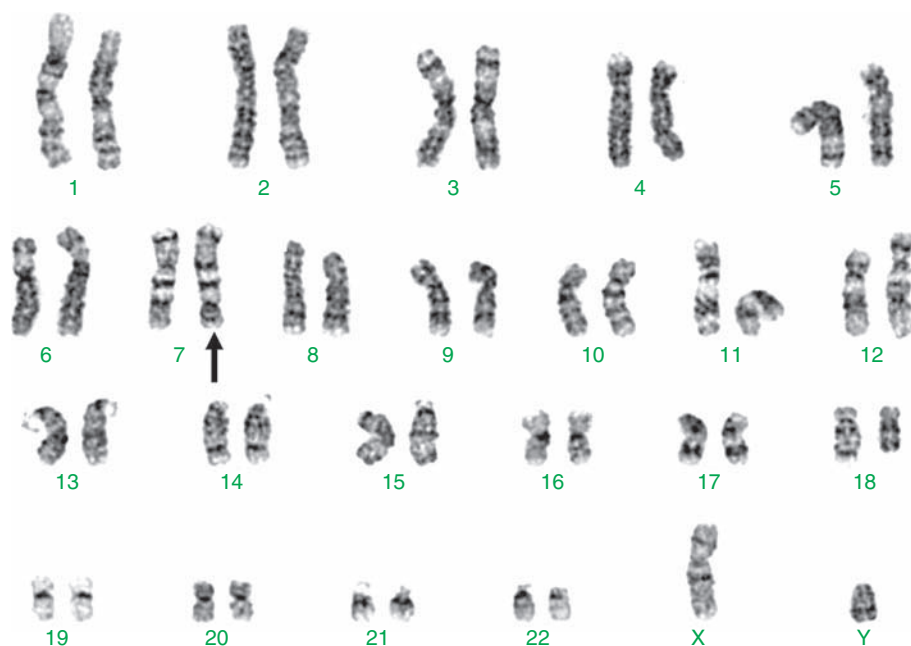


FIGURE 17.13 Karyotype of tumor cells in a patient with T-prolymphocytic leukemia demonstrating 46,XY,inv(7)(p15q35).

suggests that this virus may play a role in the pathogenesis of this disease. EBV infection has been reported in over 50% of the cases of NK-LGL leukemias reported in Japan [50]. EBV nuclear antigen 1 (EBNA-1) and EBV-encoded RNA 1 (EBER-1) have been detected in the leukemic cells by *in situ* hybridization techniques [19].

Pathology

Morphology

The peripheral blood shows an absolute lymphocytosis (usually $>10,000/\mu\text{L}$) with increased proportion of LGLs. These cells have abundant light blue cytoplasm containing azurophilic granules (see Figure 17.8). The nuclei are round, oval, or irregular and may appear pleomorphic or hyperchromatic. The nuclear chromatin is condensed and nucleoli are indistinct. The amount and the size of the azurophilic granules are variable. According to some observers, these cells are slightly larger than the normal LGLs seen in the peripheral blood [1]. Anemia is common and usually severe, and thrombocytopenia is frequent. In contrast to T-LGL leukemia, severe neutropenia is less common.

Bone marrow is almost always infiltrated by the neoplastic cells. The involvement is diffuse, focal, interstitial, or sinusoidal (Figure 17.15). The tumor cells may be mixed with normal hematopoietic cells and are sometimes difficult to detect. Scattered reactive histiocytes may be present, some showing hemophagocytosis [1, 51].

The extramedullary infiltrations may mimic *extranodal NK/T-cell lymphoma of the nasal type* and often show vascular involvement (angiocentric pattern) and areas of necrosis (see later). Most patients show splenic and hepatic involvement, often with massive hepatosplenomegaly. The leukemic infiltration in the spleen involves the red pulp and in the liver involves the portal areas and/or sinusoids [52]. Also,

the gastrointestinal tract, lymph nodes, and cerebrospinal and peritoneal fluids may be involved.

Immunophenotype

The NK-LGL leukemia cells are typically CD2+, surface CD3-, cytoplasmic CD3+, CD4-, CD7+, CD8+, CD16+, CD56+, CD57±, and TCR- (Figure 17.16) [19–21]. They also express TIA-1 and granzyme B (see Figure 17.15b and c).

Molecular and Cytogenetic Studies

The NK-cells, in contrast to T-LGLs, do not demonstrate *TCRαβ* or *TCRγδ* gene rearrangements (Figure 17.17) [53]. The evidence of EBV infection in clonal episomal form has been observed in the majority of the cases, and EBNA-1 and EBER-1 can be detected by *in situ* hybridization techniques.

Various non-random cytogenetic abnormalities have been reported in patients with aggressive NK-cell leukemia including gains of 1p, 6p, 8, 11q, 12q, 17q, 19p, 20q, and Xp, and losses of 6q (Figure 17.18), 11q, 13q, and 17p [37, 54].

Clinical Aspects

In comparison with T-LGL leukemia, NK-LGL leukemia has a more aggressive clinical course and affects younger individuals with a median age of about 40 years. The disease presents with fever, night sweats, weight loss, anemia or pancytopenia, and often massive hepatosplenomegaly [47, 55, 56]. Multiorgan failure is the major cause of death with a survival of <1 year in most instances [22]. Chemotherapy has not been effective [19]. The rare cases of EBV-negative NK-cell lymphoproliferative disorder usually have an indolent clinical course [26].

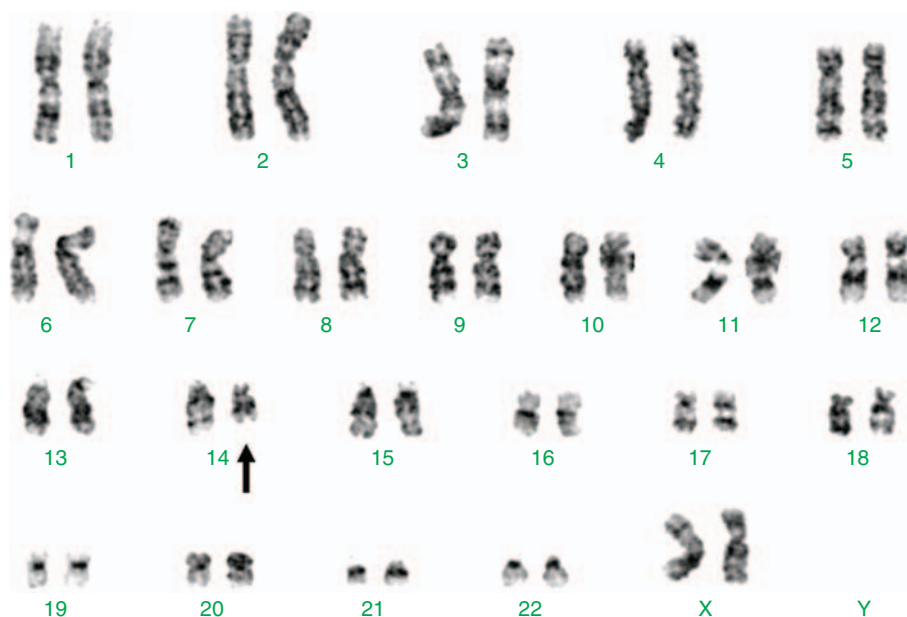


FIGURE 17.14 Karyotype of tumor cells in a patient with T-prolymphocytic leukemia demonstrating 46,XX,del(14)(q22→q24).

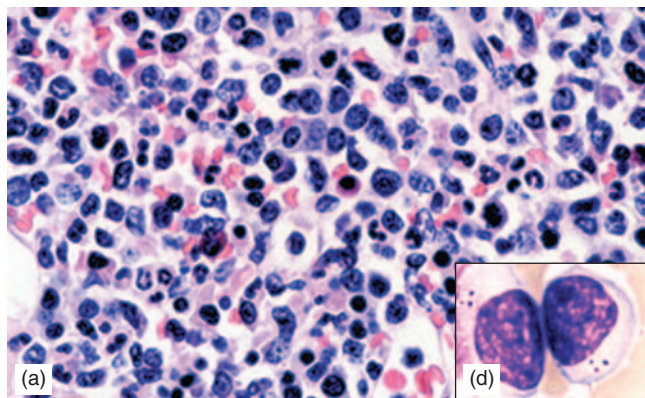


FIGURE 17.15 Bone marrow involvement in a patient with aggressive NK-cell leukemia. An interstitial atypical lymphoid infiltrate is noted (a). The tumor cells are highlighted by a TIA-1 histochemical stain (b and c). (d) Inset of (a) is a demonstration of two LGL cells by touch preparation. Adapted from Naeim F. (1997). *Pathology of Bone Marrow*, 2nd ed. Williams & Wilkins, Baltimore, by permission.

EXTRANODAL NK/T-CELL LYMPHOMA, NASAL TYPE

Extranodal NK/T-cell lymphoma, nasal type, is characterized by the strong EBV association and angiocentric infiltration, leading to vascular destruction and necrosis. The nasopharynx is the most common site of involvement,

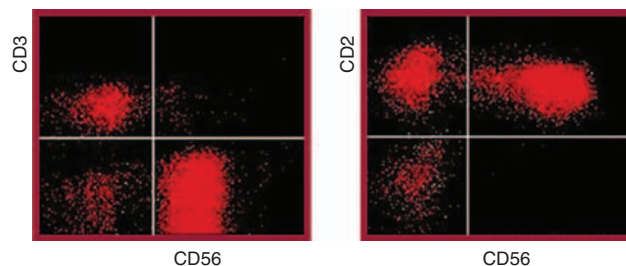


FIGURE 17.16 Flow cytometry of aggressive NK-cell leukemia. Tumor cells are positive for CD56 but lack the expression of surface CD3.

but other extranodal organs such as skin, gastrointestinal tract, testis, and soft tissues may be affected [1]. The NK-cell phenotype (CD56+, CD3−, CD8±, and EBV+) is the predominant cell type, but rare cases may demonstrate a cytotoxic T-cell phenotype (CD56−, CD3+, CD8+, and EBV+) [1, 57, 58]. This disorder was also referred to as lethal midline granuloma, polymorphic reticulosis, and malignant midline reticulosis.

Etiology and Pathogenesis

The etiology of extranodal NK/T-cell lymphoma, nasal type, is not known. The strong association of this disorder (90%) with EBV and evidence of a clonal episomal form strongly suggest a pathogenetic role for this virus [57].

Pathology

Morphology

The lymphomatous infiltrate is polymorphic with a diffuse or patchy involvement and an angiocentric and angiodestructive growth in over 85% of the cases (Figures 17.19 and 17.20) [58]. The ulceration of the overlying epithelium is common and may be associated with atypical hyperplasia of the adjacent epithelium, mimicking squamous cell carcinoma. The neoplasm consists of a mixture of small to large atypical lymphoid cells with variable amount of cytoplasm and irregular nuclei. The predominant infiltrating lymphoid cells may be large or small. The larger cells may show prominent nucleoli [49–51, 58]. Focal or confluent coagulative necrosis is common, often with the presence of apoptotic bodies [1]. Some of the tumor cells, particularly in cytologic preparations (such as touch preparation), may show cytoplasmic azurophilic granules. The lymphomatous infiltrate is often heavily admixed with inflammatory cells such as lymphocytes, plasma cells, eosinophils, and histiocytes, leading to a polymorphic pattern.

Immunophenotype

The NK-cell phenotype (surface CD3−, cytoplasmic CD3ε+, and CD56+) is the most common variant, with the vast majority of the cases being positive for CD2, TIA-1, granzyme B, and EBV [1, 60]. The NK-cell type is usually negative for other T- and NK-cell-associated markers

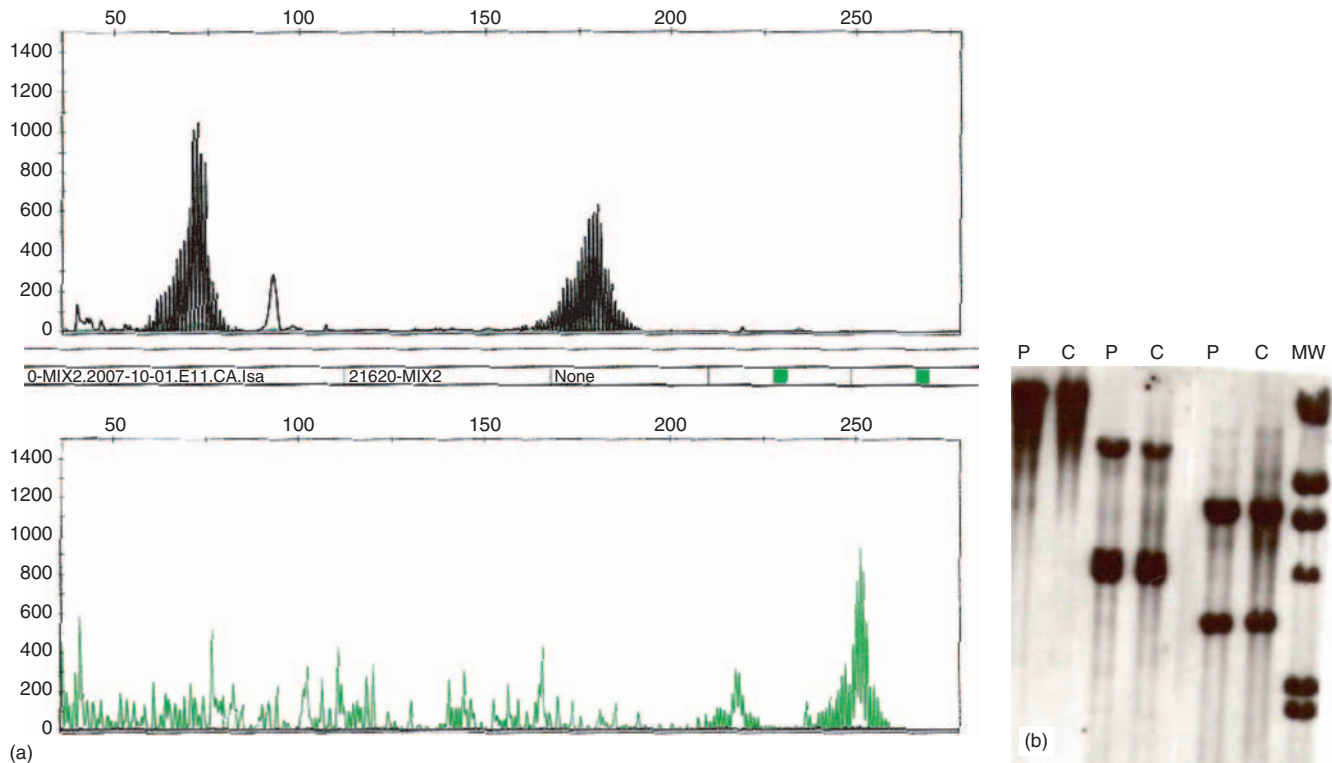


FIGURE 17.17 PCR (a) and Southern blot analysis (b) for clonal *TCR* gene rearrangements in an aggressive NK-cell leukemia, both of which are negative, showing only polyclonal or germline signals for (left-to-right, top-to-bottom) Group 1, Group 2, $V\gamma_1$, and $AltV\gamma$ by PCR, and hybridization signals for the patient (P) identical to the negative control (C) in the Southern blot (MW, molecular weight marker).



FIGURE 17.18 Karyotype of tumor cells in a patient with aggressive NK-cell leukemia demonstrating 44,X,-X,del(6)(q21),-13,del(14)(q22q24).

such as CD4, CD5, CD8, TCR ($\alpha\beta$ or $\gamma\delta$), CD16, and CD57, but occasionally may express CD7 or CD57 [1, 58, 59]. Occasional cases are CD56 $^{-}$, but are positive for CD2, cytoplasmic CD3 ϵ , EBV, TIA-1, and granzyme B.

Molecular and Cytogenetic Studies

The neoplastic cells do not demonstrate *TCR* $\alpha\beta$ or *TCR* $\gamma\delta$ gene rearrangements. The evidence of EBV infection in clonal episomal form has been observed in the vast majority

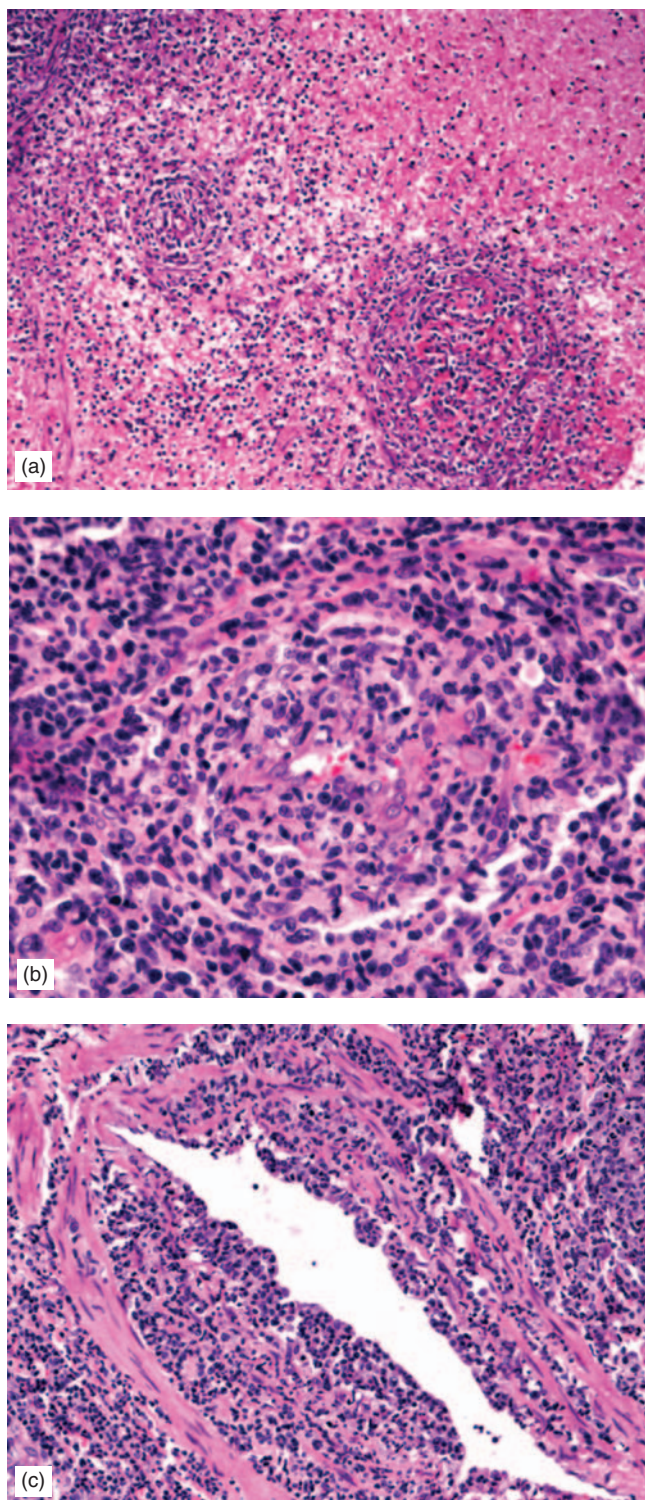


FIGURE 17.19 Extranodal NK/T-cell lymphoma, nasal type, is characterized by angiocentric and angiodestructive infiltration: (a) low power and (b) high power views. (c) The vascular infiltration may involve all the layers of the vascular wall.

of the cases, and EBNA-1 and EBER-1 can be detected by *in situ* hybridization techniques [61]. Although the *in situ* hybridization technique will demonstrate the presence of EBV RNA in essentially all of the tumor cells, proof

of clonality, in the absence of clonal *TCR* gene rearrangements, requires examination of Southern blot patterns (fingerprints) of the EBV genome [14]. PCR approaches should be used with caution, given the high frequency of latent EBV infection in the general population.

The most frequent cytogenetic finding is del(6q21-23) (Figure 17.21). Other reported non-random aberrations are i(1q), i(6p), i(7q), del(13q) +8, +X, and 11q23 rearrangements [1, 37].

Clinical Aspects

Extranodal NK/T-cell lymphomas are more prevalent in Asia and Central and South America, and predominantly affect males in the fifth decade of their age. They are clinically divided into two categories: nasal and non-nasal.

The nasal NK/T-cell lymphomas occur in the nose and the upper respiratory and oral cavities including nasopharynx, paranasal sinuses, tonsils, and larynx [49]. Symptoms are local and may include nasal obstruction, bleeding, or destruction and perforation of the hard palate [62]. These tumors are locally invasive but infrequently show distant metastasis. Less than 10% of the patients demonstrate bone marrow involvement [49, 57].

The non-nasal NK/T-cell lymphomas are often multifocal, and dissemination occurs in the early stage of the disease. The primary sites of involvement include the skin, digestive system, spleen, and testis. Nodal involvement is rare. In general, the non-nasal tumors are more aggressive than the nasal types and respond poorly to therapy. The therapeutic approaches include radiation and combination chemotherapy. The reported complete remission rate for early stages of the disease ranges from 60% to 80% for radiotherapy and from 40% to 60% for chemotherapy, but about 50% of the patients may show relapse within the first year [47, 63–65]. The expression of multidrug resistance gene may play an important role in the overall poor response to chemotherapy in a significant proportion of the patients [66].

DIFFERENTIAL DIAGNOSIS

The clinicopathologic features of T-LGL leukemia, aggressive NK-cell leukemia, and extranodal NK/T-cell lymphoma are summarized in Table 17.2. T-LGL leukemia and aggressive NK-cell leukemia should be distinguished from secondary, reactive large granular lymphocytosis. The reactive T-LGL expansions have been reported in autoimmune connective tissue disorders, inflammatory skin disorders, lymphomas, hemophagocytic syndrome, and various viral infections such as EBV, HIV, and CMV [1, 22]. Increased number of circulating NK-cells has been observed in patients with viral infection, solid tumors, MDS, and atomic bomb survivors [67]. Reactive large granular lymphocytosis is polyclonal and is not associated with chromosomal aberrations.

The differential diagnosis for extranodal NK/T-cell lymphomas of nasal type includes CD56+ T-cell lymphomas such as hepatosplenic T-cell lymphoma and other

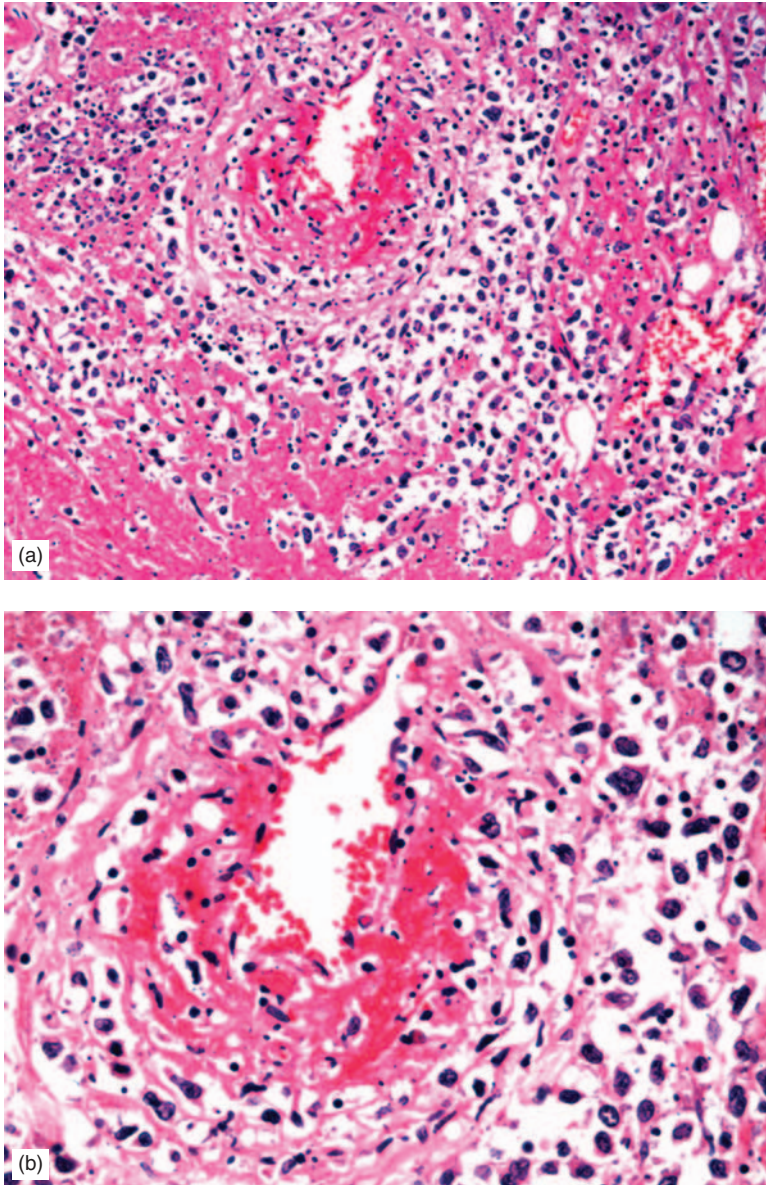


FIGURE 17.20 Extranodal NK/T-cell lymphoma, nasal type, is characterized by an angiodestructive process leading to obstruction and necrosis: (a) low power and (b) high power views.

peripheral T-cell lymphomas. The CD56+ T-cell lymphomas are negative for EBV, express surface CD3 and TCR, and show evidence of *TCR* gene rearrangement.

BLASTIC NK-CELL LYMPHOMA

This neoplasm is now considered to be of dendritic cell origin and, therefore, is discussed in Chapter 21.

FULMINANT EBV-POSITIVE T-CELL LYMPHOPROLIFERATIVE DISORDER

Fulminant EBV-positive T-cell lymphoproliferative disorder is a rare aggressive disorder presenting with fever,

hepatosplenomegaly, and pancytopenia. The patients have a clinical history of recent viral-like upper respiratory illness. The age of the patients in one report consisting of five patients ranged from 2 to 37 years and all patients died within 7 days to 8 months [68, 69]. There was an infiltration of lymphocytes in the splenic sinusoids and hepatic portal cells. These cells were positive for CD3, β F1, EBV, and TIA-1, and negative for CD56. The infiltrating T-cells in some cases were CD4+, and in other cases were CD8+, or coexpressed CD4 and CD8. The PCR studies revealed clonal *TCR* γ gene rearrangement and a deletion in the EBV latent membrane protein-1 (*LMP-1*) gene.

ADULT T-CELL LEUKEMIA/LYMPHOMA

Adult T-cell leukemia/lymphoma (ATL) is an HTLV-I-associated peripheral T-cell lymphoid malignancy often

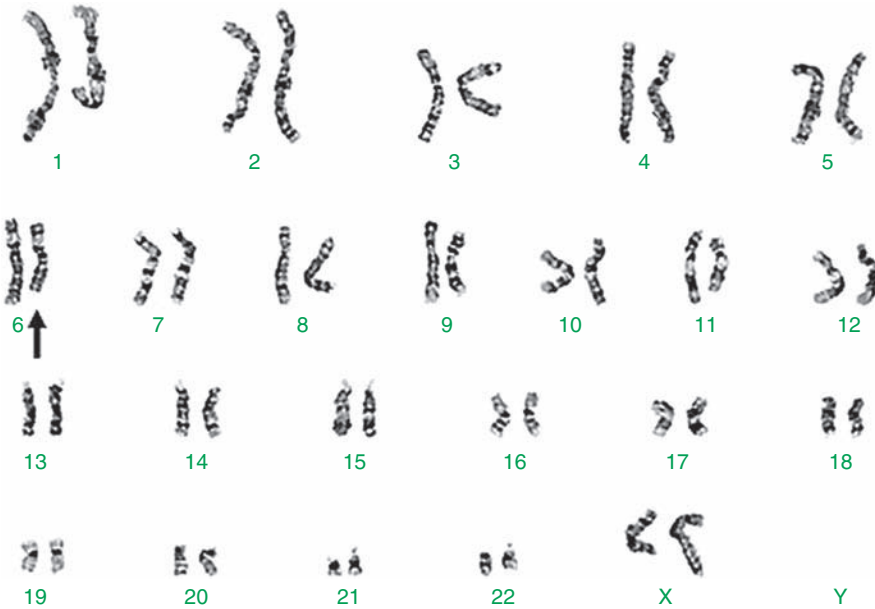


FIGURE 17.21 Karyotype of tumor cells in a patient with extranodal NK/T-cell lymphoma, nasal type, demonstrating 46,XX,del(6)(q21q23).

TABLE 17.2 Clinicopathological features of T-LGL leukemia, aggressive NK-cell leukemia, and extranodal NK/T-cell lymphoma, nasal type.

Features	T-LGL	NK-cell	Extranodal NK/T-cell
Median age (years)	60	40	50
Male:female	1	1	>1
Association	Rheumatoid arthritis	EBV	EBV
Immunophenotype	Surface CD3, CD8, TCR, CD57	CD3ε, CD56	CD3ε, CD56
Prognosis	Indolent	Aggressive	Aggressive

presenting as an acute leukemic onset and aggressive clinical course [70–72].

Etiology and Pathogenesis

HTLV-I has been implicated in the development of ATL [73–77]. A subpopulation of patients infected by HTLV-I, approximately 6% of males and 2% of females, eventually develop ATL after a long latent period [75]. The transmission of HTLV-I from infected to non-infected cells is via cell–cell interaction, which is apparently facilitated by ICAM-1 (CD56) [77]. The infected cells enter human body through three major routes: (1) sexual transmission, (2) breast feeding, and (3) parenteral transmission.

The HTLV-I *TAX* gene plays an important role in the leukemogenesis of the infected cells. The Tax protein (p40) induces proliferation and inhibits apoptosis of the HTLV-I-infected cells [78]. However, ATL cells do not always need *TAX* expression; *TAX* transcription has been

detected in only 34% of the ATL cases by RT-PCR [79, 80]. Therefore, multistep genetic and epigenetic changes are implicated in the ATL leukemogenesis. For example, mutation of *p53*, deletion of *p16*, and upregulation of *TSLC1* genes are reported in ATL [81]. The aberrant methylation of certain genes such as *MELIS* and *EGR3* is an example of epigenetic changes in ATL [82, 83]. Recent reports suggest that the HTLV-I *HBZ* gene may play an important role in the regulation of viral replication and proliferation of infected T-cells [84].

Pathology

Morphology

The neoplastic cells are pleomorphic, medium- to large-sized with variable amount of amphophilic or basophilic non-granular cytoplasm, and hyperlobated (clover leaf) or convoluted nuclei with condensed chromatin and often distinct nucleoli (Figures 17.22 and 17.23). A small proportion of blast-like cells with dispersed chromatin and prominent nucleoli are usually present. Cells resembling Reed–Sternberg cells and multinucleated anaplastic giant cells with convoluted or cerebriform nuclei may be seen in tissue infiltrations [1, 85, 86].

Bone marrow involvement is often patchy and is often associated with osteoclastic activities, leading to hypercalcemia [76, 87]. The involved lymph nodes show diffuse infiltration with effacement of nodal architect and proliferation of endothelial venules. The skin infiltration usually involves the upper dermis, with frequent epidermal involvement and formation of tumor cell aggregates resembling Pautrier microabscesses [1, 86].

Immunophenotype

The neoplastic cells are typically of T-helper phenotype expressing CD4 and T-cell-associated markers CD2, CD3, and CD5 (Figure 17.24) [85, 86]. The majority of the cases

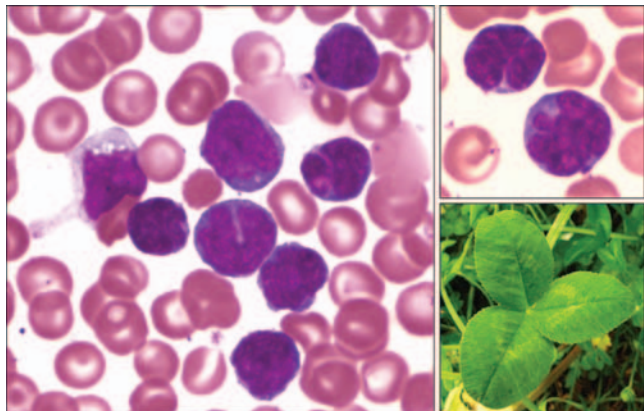


FIGURE 17.22 Adult T-cell leukemia/lymphoma. Blood smears showing atypical medium- to large-sized cells with amphophilic or basophilic non-granular cytoplasm and hyperlobated (clover leaf) nuclei.

lack CD7 expression but are positive for CD25 and CD52 [88, 89]. Occasional cases may be CD4[−]/CD8⁺ or CD4⁺/CD8⁺. TIA-1 and granzyme B are negative. The anaplastic large cells may express CD30, but they are negative for anaplastic lymphoma kinase (ALK) [1].

Cytogenetic and Molecular Studies

There is no distinct karyotypic or molecular abnormality in ATL. Cytogenetic analysis often shows a complex karyotype, particularly in the leukemic forms. Recurrent abnormalities include +3, +7, +21, monosomy X, loss of chromosome Y, and abnormalities of chromosomes 6 and 14q (at 14q11.2 and 14q32 breakpoints) (Figure 17.25) [85, 90, 91].

The neoplastic cells demonstrate *TCR* gene rearrangements with clonally integrated HTLV-I [92]. The detection and clonal pattern analysis of HTLV-I are quite specialized and are available only in selected reference laboratories [93]. The deletion of *p16* (multiple tumor suppressor 1) gene and the mutation of *p53* gene have been reported [94].

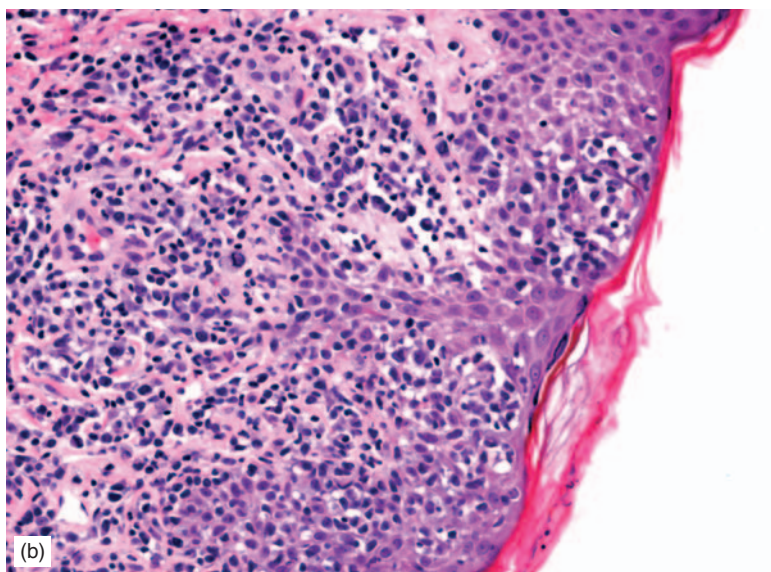
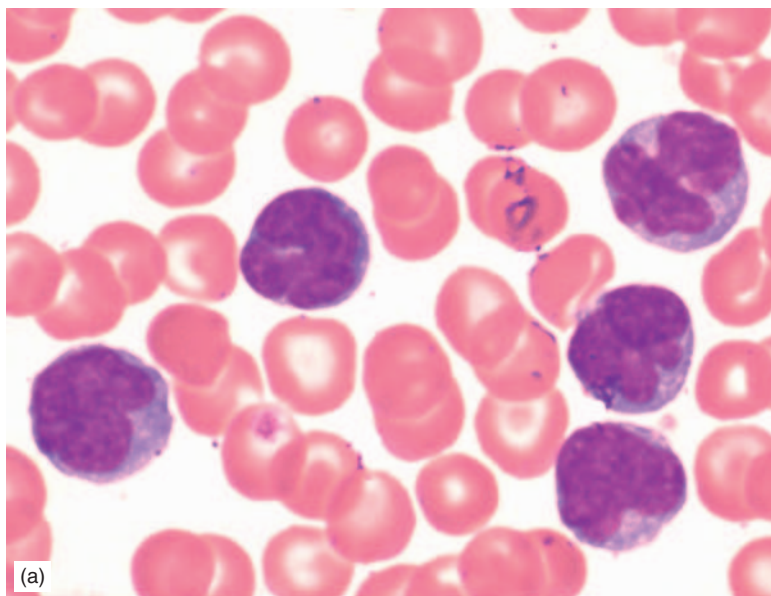


FIGURE 17.23 Adult T-cell leukemia/lymphoma. (a) Blood smear demonstrating atypical lymphocytes with lobulated nuclei. (b) Skin biopsy section showing a heavy lymphoid infiltrate in the upper dermis and epidermis.

Clinical Aspects

The geographic areas with the highest prevalence of HTLV-I include Japan, Africa, Caribbean islands, South America, and southern part of the United States [76, 95, 96]. Patients are adults with a median age of about 50 years with male:female ratio of about 3:2 [1]. Clinical symptoms may include hypercalcemia, lytic bone lesions, cutaneous lesions

simulating mycosis fungoides (MF), lymphadenopathy, pulmonary lesions, and hepatosplenomegaly. Hypercalcemia is observed in over 70% of the cases during the clinical course, which appears to be due to osteoclastic proliferation and increased bone resorption. ATL cells have been shown to express receptor activator of nuclear factor-kappaB (RANK) ligand, which plays a role in the differentiation of hematopoietic precursors to osteoclasts [97]. There are four types of clinical presentation [72, 76, 85, 94, 98]:

1. Acute onset which is the most common type and occurs in approximately 60% of cases. It has an aggressive clinical course with 4-year survival rate of 5–12%.
2. The lymphomatous type representing about 20% of the cases characterized by prominent lymphadenopathy and no blood involvement but also aggressive clinical course.
3. The chronic type consisting of about 15% of the cases with skin lesions and absolute lymphocytosis, but no hypercalcemia.
4. The smoldering type representing 5% of the cases with normal blood lymphocyte counts and <5% circulating neoplastic cells and frequent skin or pulmonary lesions. There is no hypercalcemia.

Approximately 25% of the cases of chronic or smoldering types eventually progress to an acute phase. This transition is often associated with specific changes on gene expression profiling [94, 99].

The clinical outcome in majority of the cases is very poor with a median survival of <1 year despite advances in chemotherapy [100]. Combination chemotherapies such as cyclophosphamide, adriamycin, vincristine, and prednisone (CHOP), nucleoside analogs, topoisomerase inhibitors, interferon, and zidovudine are among the therapeutic chances [76].

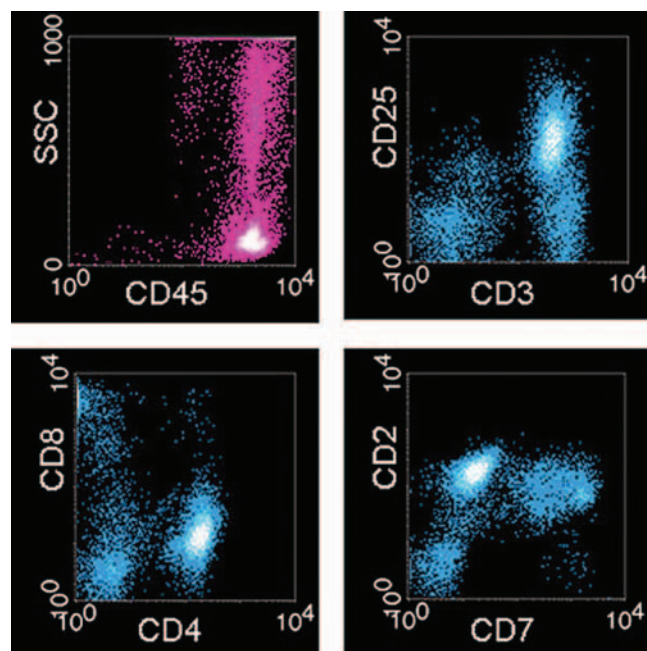


FIGURE 17.24 Adult T-cell leukemia/lymphoma. Flow cytometry demonstrating a population of helper T-cells (CD3+, CD4+) with loss of CD7 expression. The tumor cells are also CD2+ and CD25+.

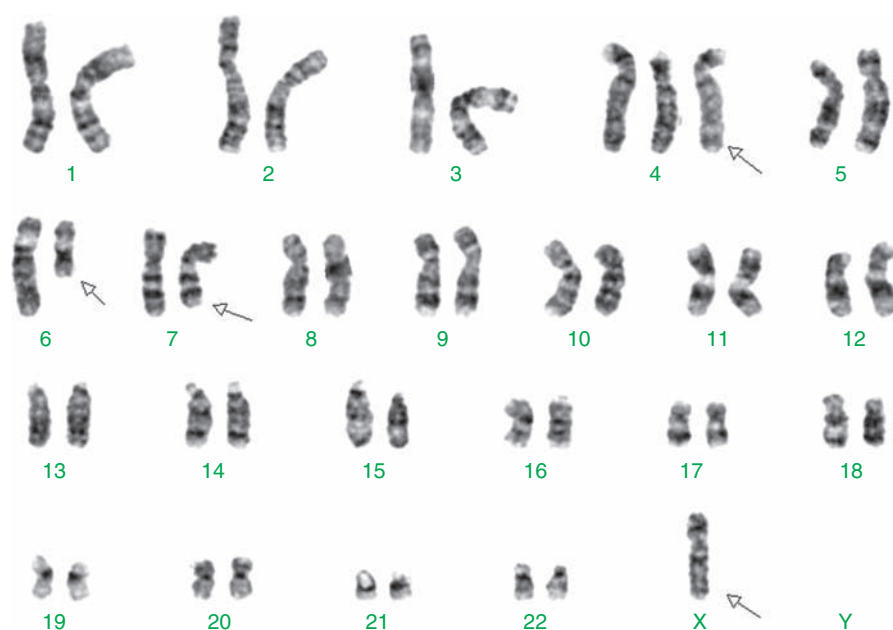


FIGURE 17.25 Karyotype of tumor cells in a patient with ATL demonstrating 46,X,-X,+4,del(6)(q15),del(7)(q35).

Differential Diagnosis

The neoplastic cells in peripheral blood and bone marrow smears may mimic Sezary cells and may share the same immunophenotypic features (expressing CD4 and pan-T-cell markers except CD7). In general, nuclear convolution in Sezary cells is more delicate and finer than that in the neoplastic ATL cells. In all ATL cases, the neoplastic cells are positive for HTLV-I, whereas the majority of the patients with SS are HTLV-I negative. Also, the median age for ATL is lower than that of MF/SS. Hypercalcemia and lytic bone lesions are frequent findings in ATL but absent in MF/SS.

The tissue infiltrates in ATL may contain anaplastic large cells and/or Reed–Sternberg-like cells mimicking anaplastic large cell lymphoma (ALCL) or Hodgkin lymphoma.

MYCOSIS FUNGOIDES AND SEZARY SYNDROME

Mycosis fungoides (MF) is a cutaneous peripheral T-cell lymphoma (CTCL) characterized by an indolent course and skin manifestations ranging from patches to plaques and tumor formation. Sezary syndrome (SS) is the leukemic and erythrodermic variant of MF with the presence of neoplastic cells in the peripheral blood.

Etiology and Pathogenesis

The etiology and pathogenesis of MF/SS are not known. An infectious etiology has been suggested [101]. For example, a *Borrelia burgdorferi*-specific sequence has been detected in 205 of the skin samples of patients with MF [102]. Coinfection of HIV-1 and HTLV-II has been reported in three patients with MF-like disorders [103, 104]. The possible association between HTLV-I and MF/SS is controversial [105–107].

Pathology

Morphology

Skin biopsies demonstrate an infiltrate of atypical lymphoid cells in the upper dermis and epidermis. These atypical cells are usually medium to large size with variable amount of cytoplasm and convoluted (cerebriform) nuclei. The intraepidermal lesions characteristically consist of small aggregates of atypical cells referred to as “Pautrier microabscesses” (Figures 17.26 and 17.27). In one study, in addition to the Pautrier abscesses, which were observed in 38% of the cases, the following morphologic features were frequently observed: haloed lymphocytes, exocytosis, epidermal lymphocytes larger than dermal lymphocytes, and lymphocytes aligned within the basal layer [1, 108–110]. In occasional cases, referred to as *pagetoid reticulosis*, the lymphoid infiltrates are strictly epidermal. Pagetoid reticulosis is usually localized and has an excellent prognosis [1, 111]. The epidermal infiltrate is patchy, diffuse, or band-like and

is often associated with reactive inflammatory cells such as lymphocytes and eosinophils [1].

Lymphadenopathy is frequent in advanced cases (30–40%) and is divided into three categories as follows:

1. Dermatopathic lymphadenitis with scattered cerebriform lymphocytes, but no clusters.
2. Focal effacement of nodal architecture with clusters of atypical cerebriform lymphocytes, primarily in the paracortical regions.
3. Complete effacement of nodal architecture with diffuse infiltration of atypical cerebriform lymphocytes.

SS is considered a variant of MF associated with the presence of atypical cerebriform lymphocytes (Sezary cells) in the peripheral blood. The Sezary cells have a variable amount of non-granular cytoplasm and show the characteristic delicately convoluted, cerebriform nucleus with condensed chromatin and inconspicuous nucleoli (Figures 17.26c and 17.28). These cells may vary in size with the smaller forms referred to as Lutzner cells. The diagnosis of SS according to the International Society for Cutaneous Lymphoma (ISCL) is based on one or more of the following [112]:

- A. An absolute Sezary cell count of $\geq 1,000$ cells/ μ L.
- B. A CD4:CD8 ratio of ≥ 10 caused by an increase in circulating T-lymphocytes, or an aberrant loss of pan-T-cell markers.
- C. Lymphocytosis with the evidence of a T-cell clone by the Southern blot or PCR.
- D. A chromosomal abnormal T-cell clone.

In the cases of B, C, and D, at least 5% of the circulating lymphocytes should demonstrate characteristic morphologic features of the Sezary cells.

Immunophenotype

The neoplastic MF/SS cells are of helper T-cell phenotype (CD4+) and express CD45 and pan-T-cell markers CD2, CD3, and CD5, and TCR β , but are usually negative for CD7, CD8, CD26, TRA-1, and granzyme B (Figures 17.27 and 17.29) [110, 113, 114]. The MF/SS cells are usually CD158+ and may express the IL-2 receptor- α (CD25). Atypical patterns such as CD4-/CD8- or CD4-/CD8+ phenotypes are rare.

Cytogenetic and Molecular Studies

Patients with cutaneous T-cell lymphoma commonly show a wide variety of clonal or non-clonal chromosomal aberrations in their blood or skin. Standard karyotyping studies of CTCL are difficult, as the malignant cells respond poorly to mitogens needed for inducing visible, analyzable mitotic chromosomes for G-banding staining. However, no recurrent or specific abnormality has been found in CTCL, leading to a hypothesis of genetic instability.

The cytogenetic studies in MF/SS have been largely performed on blood lymphocytes and revealed a large spectrum of chromosomal abnormalities, both numerical and structural. In one study by Whang-Peng *et al.* [115] the chromosomes most often involved in structural abnormalities

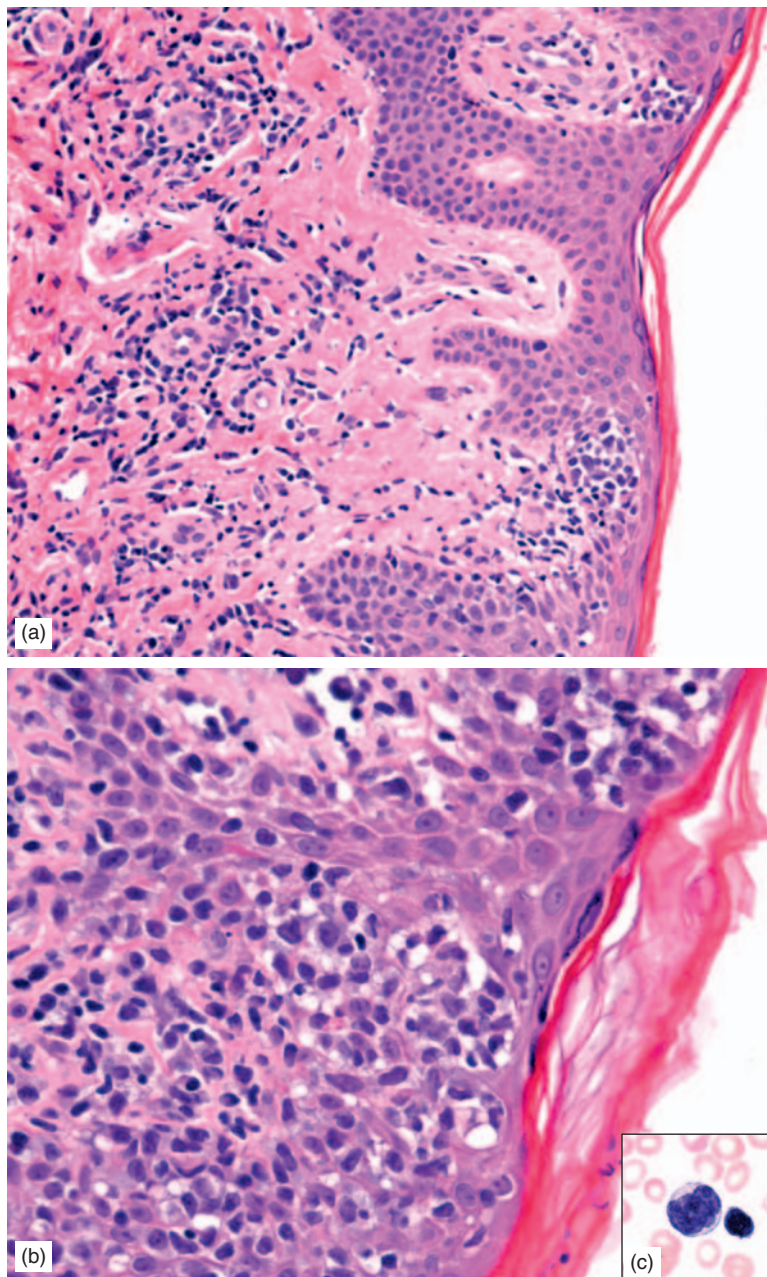


FIGURE 17.26 Mycosis fungoides/Sezary syndrome. Section of skin demonstrating an infiltrate of atypical lymphoid cells in the upper dermis and epidermis with Pautrier microabscesses: (a) low power and (b) high power views. Blood smear (c) showing a Sezary cell with convoluted nucleus.

of 1, 6, 7, 4, 9, 10, 12, 14, 15, and 17, and numerical abnormalities of chromosomes 11, 21, 22, 8, 9, 15, 16, and 17, respectively, in order of frequency. Cytogenetic abnormalities were observed prior to malignancy observed by histology and were suggested to have a significant diagnostic and prognostic value. CGH studies revealed loss of chromosomal region at bands 10q25-q26 and 13q21-q22 and gains (amplifications) in chromosomes 8 and 17q21-q25 in SS. DNA content analysis may show evidence of aneuploidy in up to one-third of the patients (Figure 17.30).

The MF/SS cells show *TCR* gene rearrangement. Additional *Her2/neu* gene copies and inactivation of *CDKN2A/p16* have been reported in MF/SS cells [116, 117]. In one study, real-time PCR studies demonstrated overexpression of five genes, *STAT4*, *GATA-3*, *PLS3*, *CD1D*, and *TRAIL*, by the circulating tumor cells in patients with SS [118].

Clinical Aspects

MF/SS comprises <1% of all non-Hodgkin lymphomas, but it is the most common primary cutaneous T-cell lymphoma accounting for about 45% of lymphomas present in the skin [119–121]. The peak age is 55–60 years, with a male:female ratio of about 2:1. MF usually presents as indolent cutaneous erythematous scaly patches or plaques, often with pruritus, mimicking common skin disorders such as eczema or psoriasis [101]. These lesions may wax and wane for many years and may eventually progress to cutaneous tumor formation, erythroderma, and the infiltration of the neoplastic cells into the circulation (SS). Extracutaneous involvement is relatively uncommon in the early stages of the disease but becomes more frequent in the advanced stages, comprising 8% in the plaque stage compared to 30–40% in the erythrodermatous stage [101, 119, 120].

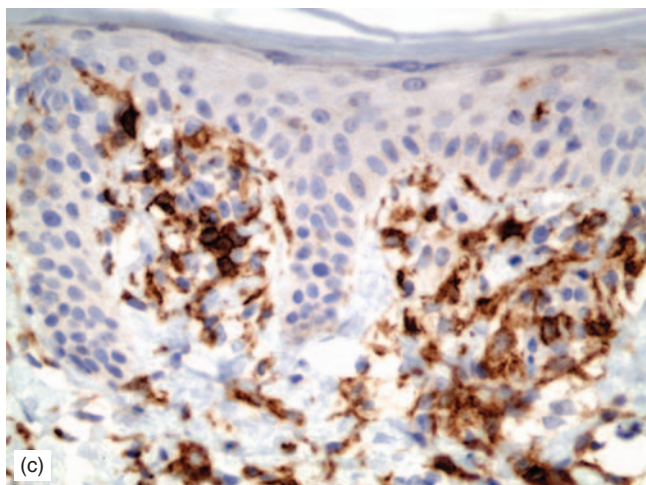
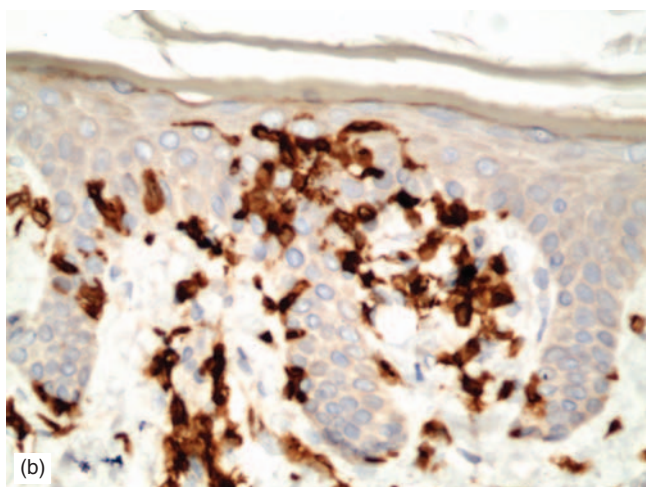
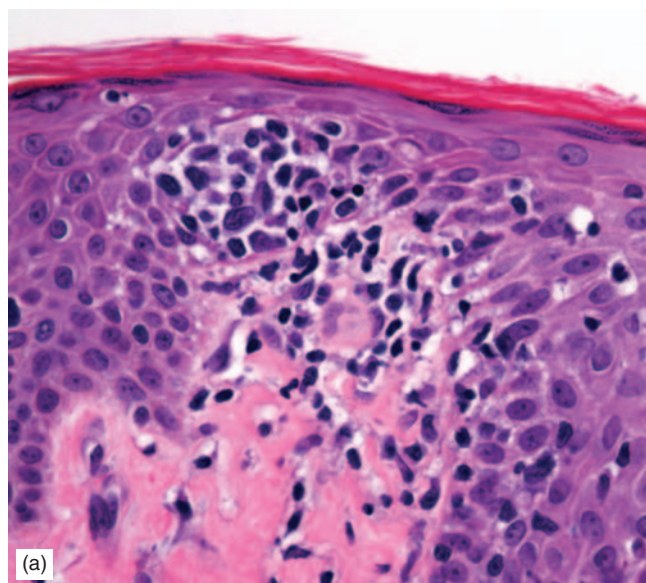


FIGURE 17.27 Mycosis fungoides. Section of skin demonstrating an infiltrate of atypical lymphoid cells in the upper dermis and epidermis (Pautrier microabscess) (a). These cells are positive for CD3 (b) and CD4 (c) by immunohistochemical stains.

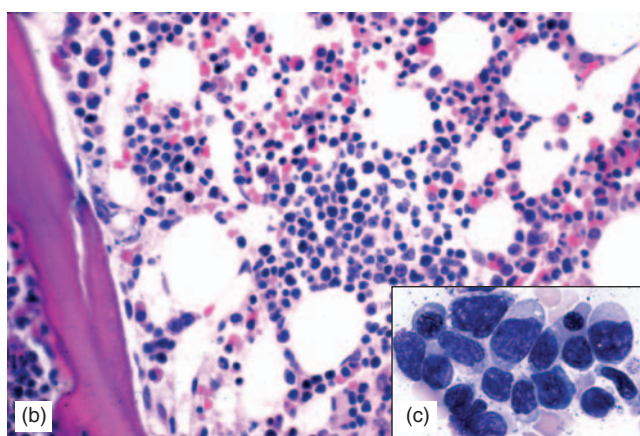
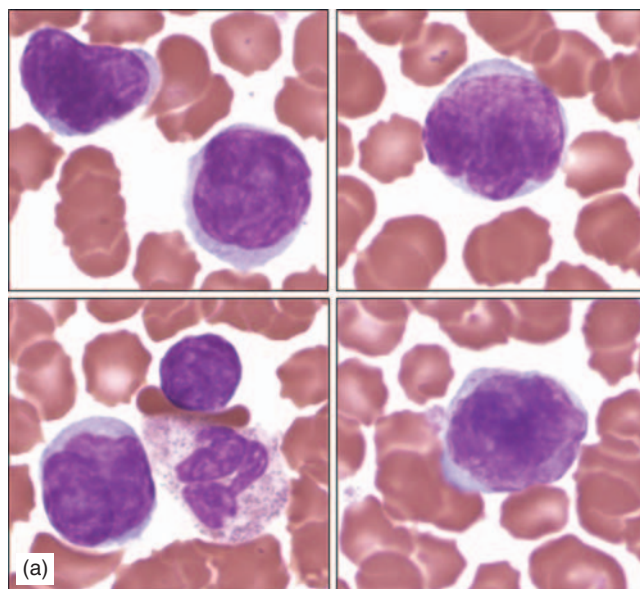


FIGURE 17.28 Mycosis fungoides/Sezary syndrome. Blood smear several Sezary cells with convoluted nuclei demonstrating (a). Bone marrow biopsy section (b) and smear (c) showing an aggregate of atypical lymphocytes with irregular nuclei. Adapted from Naeim F. (1997). *Pathology of Bone Marrow*, 2nd ed. Williams & Wilkins, Baltimore, by permission.

The regional lymph nodes are the most frequent sites of involvement followed by lungs, spleen, and liver.

The clinical staging is based on the extent of cutaneous lesions (T) and the involvement of the lymph nodes (L), viscera (M), and blood (B) (see Table 17.3) [121]. In the majority of the cases, cutaneous lesions are at stage T1 (20–25%) or T2 (35–40%). The overall 5-year survival for MF has been reported as 87% compared to 33% for SS [109, 122]. Therefore, transformation from MF to SS is an indicative of poor prognosis [122–124]. Transformation to CD30+ large cell lymphoma is relatively frequent. In two large studies of patients with MF, the cumulative probability of transformation to large cell lymphoma was 39% in 12 years, with a median time interval of 1–6.5 years after diagnosis of MF [125, 126]. The criteria for transformation were the formation of microscopic nodules of large neoplastic cells or >25% large cells in the neoplastic infiltrate [126]. The elevation of serum lactate dehydrogenase (LDH) and β 2-microglobulin was predictive of transformation.

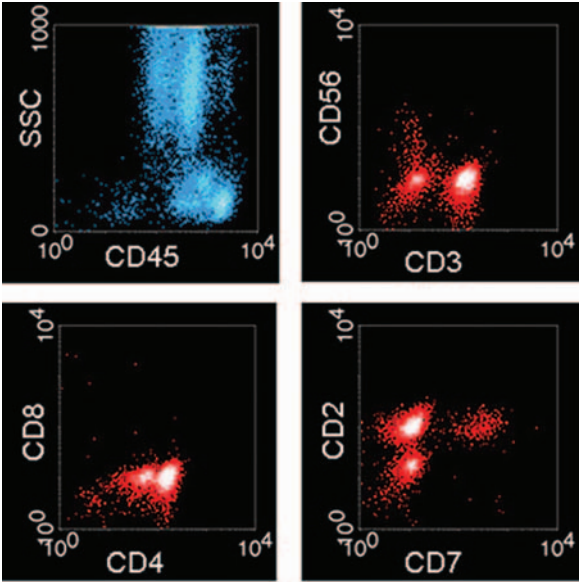


FIGURE 17.29 Mycosis fungoides/Sezary syndrome. Flow cytometry demonstrating a population of helper T-cells (CD3+, CD4+) with the loss of CD7 expression. The tumor cells are also positive for CD2.

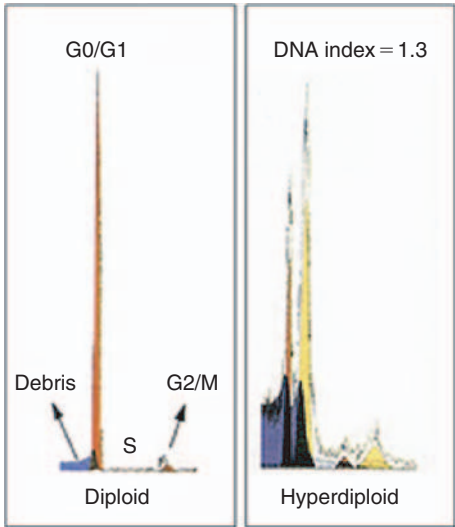


FIGURE 17.30 Mycosis fungoides/Sezary syndrome. DNA content analysis by flow cytometry demonstrating a hyperdiploid population (right yellow).

Transformation to CD30+ large cell lymphoma is associated with an aggressive clinical course. In one study, the median survival was 3 years in transformed MF patients compared to 14 years in untransformed patients [126].

Treatment includes a broad spectrum of options. The topical therapeutic measures, such as nitrogen mustard, carmustine, electron beam therapy, and phototherapy, are used for the early stages of the disease. Systemic chemotherapy, alone or in combination with topical therapy, is used in the advanced stages of the disease [101, 127]. Purine and pyrimidine analogs are the primary chemotherapy agents.

TABLE 17.3 Clinical staging for MF.*

<i>Skin (T)</i>
T1: Limited involvement; patches/plaques <10% of the total skin surface
T2: Generalized involvement; patches and plaques >10% of the total skin surface
T3: Cutaneous tumors
T4: Generalized erythroderma
<i>Lymph nodes (N)</i>
N0: No enlarged lymph node
N1: Lymphadenopathy with no evidence of histologic neoplastic involvement
N2: No lymphadenopathy but evidence of histologic involvement
N3: Lymphadenopathy and histologic involvement
<i>Viscera (M)</i>
M0: No visceral involvement
M1: Histologically confirmed visceral involvement
<i>Blood (B)</i>
B0: Circulating Sezary cells <5% of the lymphocytes
B1: Circulating Sezary cells >5% of the lymphocytes

*Adapted from the *AJCC Cancer Staging Manual*, 6th ed. Springer-Verlag, New York, 2002.

Differential Diagnosis

The differential diagnosis of MF includes a garden variety of benign reactive skin disorders, such as psoriasis, eczema, parapsoriasis, drug reactions, contact dermatitis, and photo-dermatitis. These distinctions can be quite challenging and often settled only by the results of *TCR* gene rearrangement studies. As the most referred skin biopsies will be paraffin-embedded, PCR analysis is the primary approach for these lesions, since formalin-fixed tissue does not yield DNA of high enough quality for Southern blot analysis to be reliable. However, it is in this setting that the potential for false-positive results due to spurious amplification of a small number of T-lymphocytes in the specimen (pseudoclonality) comes to the fore. In our laboratory, we include a disclaimer to this effect when the skin biopsy contains only scattered or scant T-lymphocytes and/or when we see an isolated clonal spike in the PCR profile in the absence of any polyclonal background signal; this is a hint that the signal may be artifactual (see Figure 17.4). MF should be distinguished from other primary cutaneous lymphomas. It shares overlapping morphologic and immunophenotypic features with ATL. ATL occurs in a younger age group and is associated with HTLV-I (Table 17.4). The neoplastic cells in a minority of T-PLL cases are Sezary-cell like (see T-PLL in this chapter). The T-PLL cells are usually CD7+ (Table 17.4).

HEPATOSPLENIC T-CELL LYMPHOMA

Hepatosplenic lymphoma is a rare extranodal peripheral T-cell lymphoma of cytotoxic type characterized by sinusoidal infiltration of liver, spleen, and bone marrow.

TABLE 17.4 Clinicopathological features of T-prolymphocytic leukemia (T-PLL), adult T-cell leukemia/lymphoma (ATL), and mycosis fungoides/Sezary syndrome (MF/SS).

Features	T-PLL	ATL	MF/SS
Median age (years)	>50	50	55–60
Male:female	?	1.5	2
Association	<i>ATM</i> *	<i>HTLV-I</i>	<i>HTLV-I</i> ?
Skin involvement (%)	15	15	100
Immunophenotype	CD3+, CD4+, CD7+, some CD4+/CD8+	CD3+, CD4+, CD7–	CD3+, CD4+, CD7–, CD26–
Overall prognosis	Aggressive	Aggressive	Indolent

*Ataxia-telangiectasia gene.

In most reported cases, the neoplastic cells represent the TCR $\gamma\delta$ T-cell subtype, though rare cases express TCR $\alpha\beta$ [1, 128–133].

Etiology and Pathogenesis

The etiology and pathogenesis of hepatosplenic T-cell lymphoma are not known. Frequent report of this disorder in patients with immune-compromised conditions, such as immunosuppressive therapy for solid organ transplantation, autoimmune disorders, and malaria, raises the possibility of an association between impairment of the immune system and development of hepatosplenic lymphoma. All of these conditions have been associated with the expansion of $\gamma\delta$ T-cells, probably secondary to chronic antigenic stimulation [1, 134–136]. The proliferation of $\gamma\delta$ T-cells in immune-compromised conditions may represent the initial step of a multistep process in the pathogenesis of hepatosplenic lymphoma.

Pathology

Morphology

Liver infiltration is sinusoidal with various degrees of portal tract involvement (Figure 17.31). Splenomegaly is a common feature. The pattern of infiltration in the spleen is diffused with the involvement of the red pulp (Figure 17.32). The neoplastic lymphoid cells are usually monomorphic, with moderate amount of cytoplasm; small to medium size, round to slightly irregular nuclei; condensed chromatin; and inconspicuous nucleoli [132, 133]. Mitotic figures are usually infrequent. Occasional cases may show a highly pleomorphic cell population. The bone marrow involvement is reported in 75–100% of the cases. The bone marrow infiltration is often subtle and sinusoidal (Figure 17.33). Occasionally, there is an increased number of histiocytes with features of hemophagocytosis [133]. Fulminant leukemic picture is rare, but scattered abnormal cells may be seen in the peripheral blood (Figure 17.33a). The lymph node

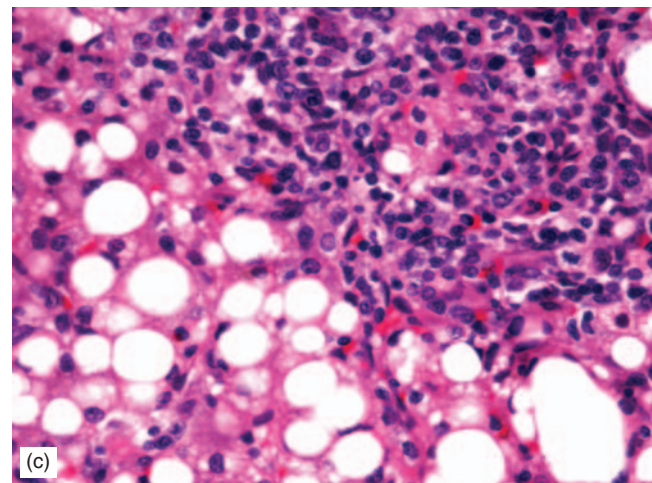
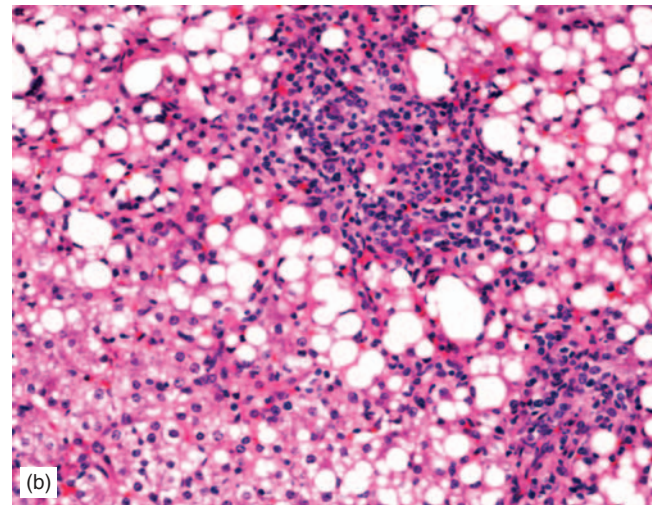
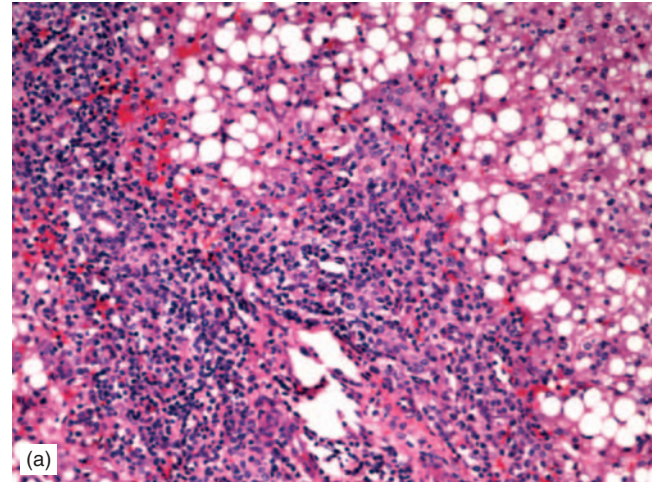


FIGURE 17.31 Hepatic involvement with hepatosplenic T-cell lymphoma. Patchy lymphoid infiltration is evident in a liver with fatty degeneration: (a) low power, (b) intermediate power, and (c) high power views.

involvement is rare. There are other variants of $\gamma\delta$ T-cell lymphomas, which mostly involve extranodal tissues, such as skin, subcutaneous tissue, or intestine, which are not considered as hepatosplenic lymphoma [1, 137].

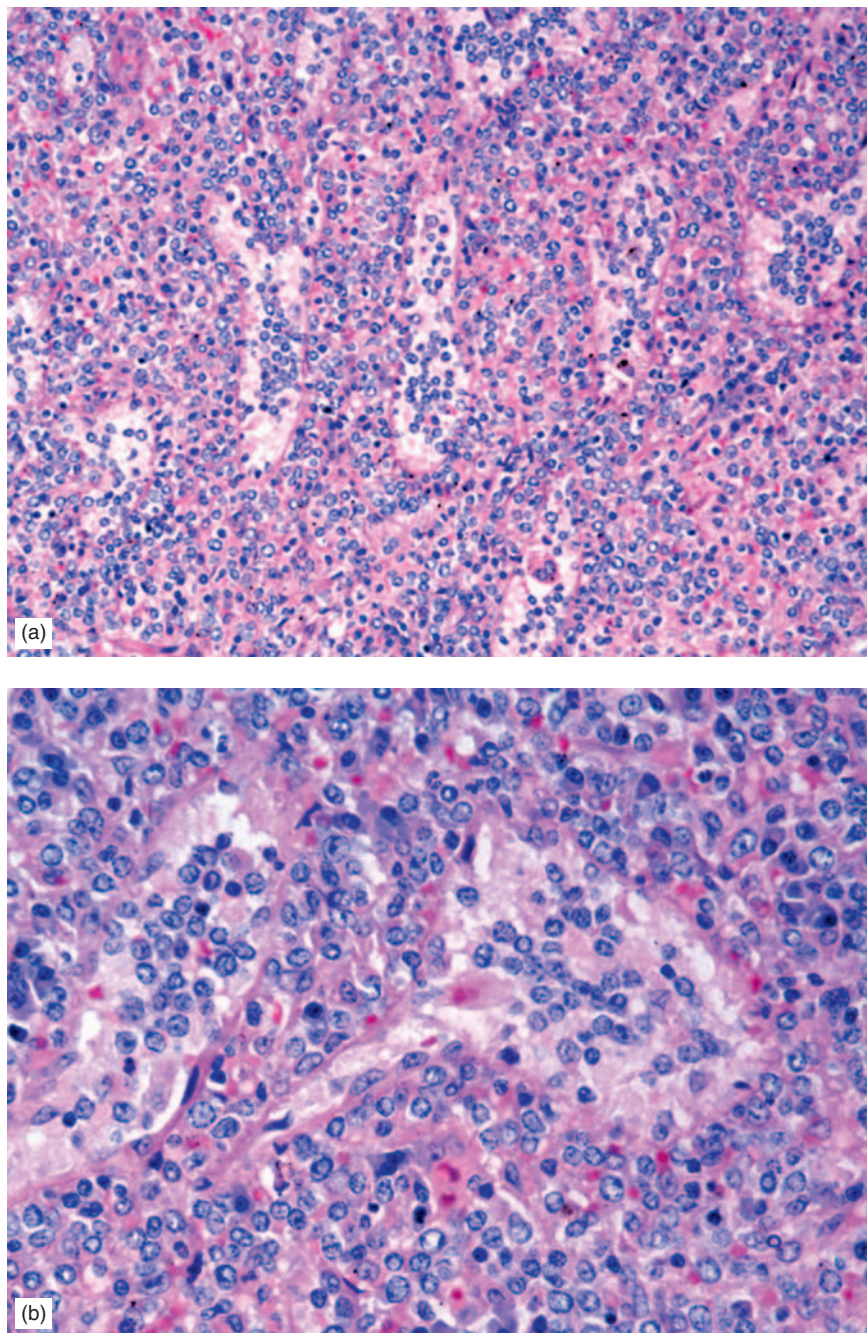


FIGURE 17.32 Hepatosplenic T-cell lymphoma with the involvement of splenic red pulp and sinuses: (a) low power and (b) high power views.

Immunophenotype

The most common immunophenotypic features of the neoplastic cells are $\gamma\delta$ TCR+(γ TCR1), CD2+, CD3+, CD56+, TIA-1+, CD7 \pm , CD4–, CD5–, CD8–, and granzyme B– (Figures 17.33 and 17.34). Rare cases may demonstrate TCR $\alpha\beta$ or express CD8 [138, 139].

Molecular and Cytogenetic Studies

The neoplastic cells typically show *TCR* gene rearrangement by Southern blotting or PCR techniques. The *in situ* hybridization studies for EBV are negative [1]. The most frequent cytogenetic abnormalities are isochromosome 7q and

trisomy 8 [133]. Isochromosome 7q is currently viewed as a pathognomonic genetic alteration in hepatosplenic T-cell lymphoma and can therefore serve as a diagnostic tool for this entity (Figure 17.35) [140, 141]. Also, del(13)(q12q14) has been reported in patients with hepatosplenic lymphoma (Figure 17.36) [131].

Clinical Aspects

Hepatosplenic T-cell lymphoma is a rare disease, accounting for about 5% of all peripheral T-cell lymphomas. The median age is about 35 years with the male:female ratio

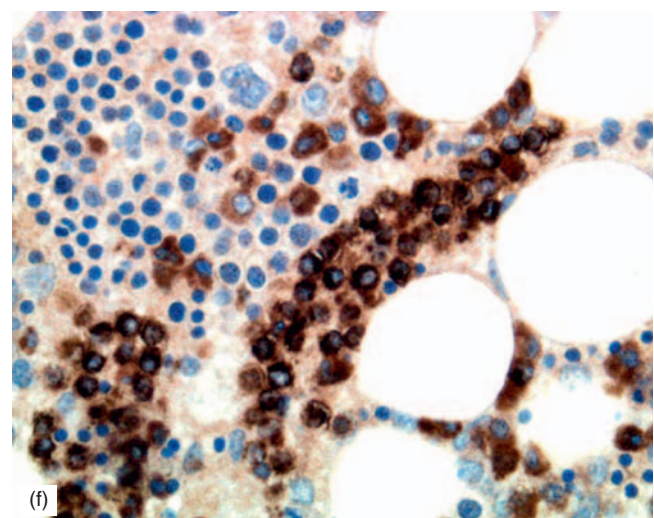
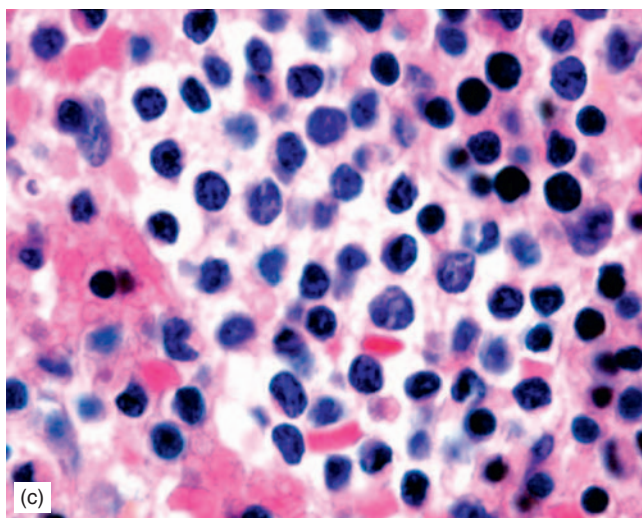
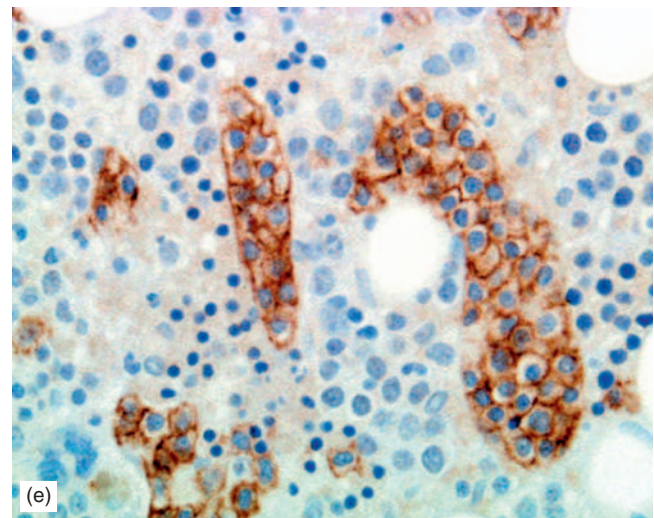
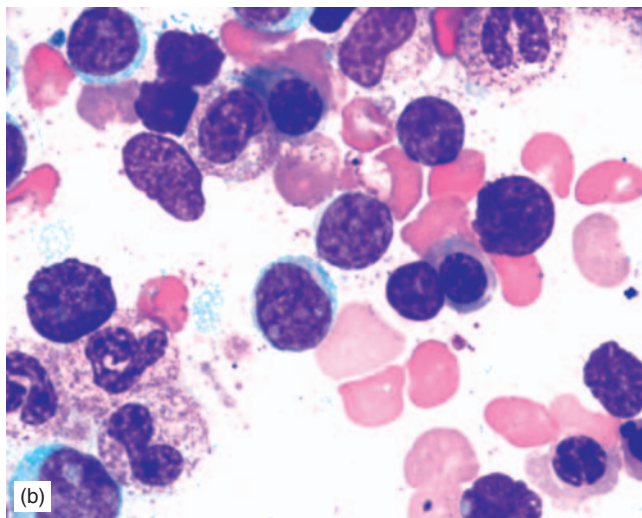
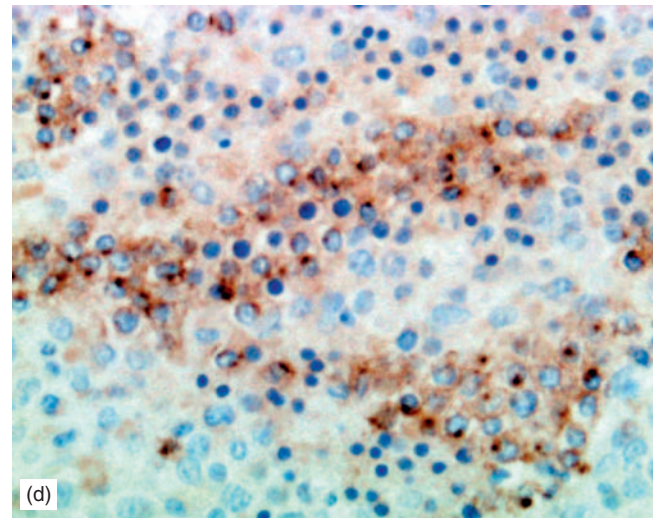
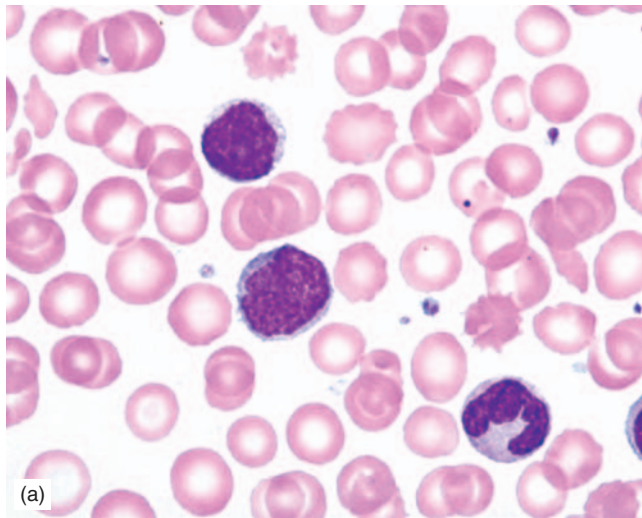


FIGURE 17.33 Hepatosplenic T-cell lymphoma. Blood (a) and bone marrow (b) smears showing atypical lymphocytes. Bone marrow involvement is often sinusoidal (c) demonstrated by accumulation of CD3+ (d), CD56+ (e), and TIA-1+ (f) cells in the sinusoids.

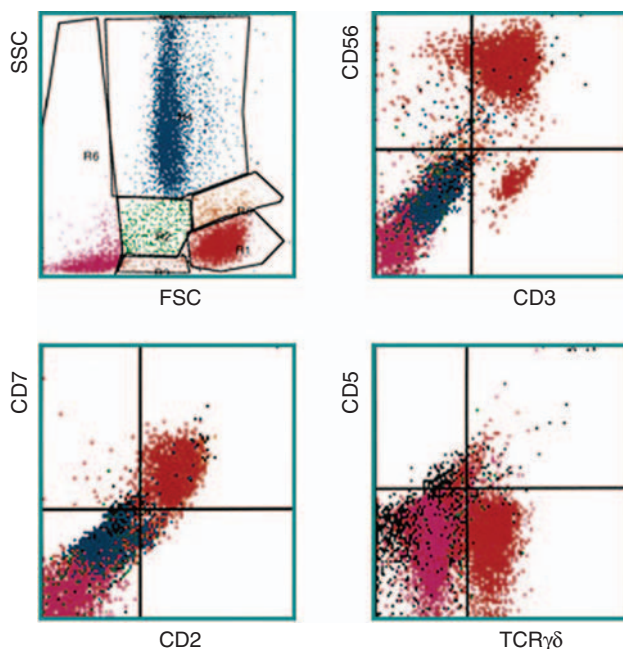


FIGURE 17.34 Flow cytometric studies demonstrating the common $\gamma\delta$ variant of hepatosplenic T-cell lymphoma with the expression of CD2, CD3, CD7, and CD56. CD5 is negative.

of around 3:1 [133, 140]. Splenomegaly and thrombocytopenia are commonly followed by hepatomegaly, anemia, and leukopenia. Lymphadenopathy and other extranodal involvements are rare [140].

Hepatosplenic T-cell lymphoma has an aggressive clinical course with a median survival of about 16 months [133]. Combination chemotherapy is the frequent therapeutic approach, often with unsatisfactory results [133].

Differential Diagnosis

The differential diagnosis includes NK-cell lymphoproliferative disorders, because of overlapping morphologic pattern (sinusoidal involvement) and immunophenotypic features (CD56 and TIA-1 expression). However, neoplastic cells of hepatosplenic lymphoma are CD3+ and granzyme B-, and in many cases demonstrate isochromosome 7q. The T-LGL leukemia cells are CD3+ and may occasionally express *TCR* $\gamma\delta$, whereas lymphoid cells of hepatosplenic lymphoma are CD5-, CD56-, and CD57+ [138].

ENTEROPATHY-TYPE T-CELL LYMPHOMA

Enteropathy-type T-cell lymphoma is a rare intraepithelial T-cell intestinal lymphoma consisting of a polymorphic lymphoid infiltrate of medium to large atypical lymphocytes [1, 142–144].

Etiology and Pathogenesis

The etiology and pathogenesis of this disorder are not known. There is a strong association with celiac disease with

the evidence of serologic markers such as positive antigliadin antibodies and HLA-DQA1, B1, and HLA-DRB1 types [145, 146]. Some cases in South and Central America have been associated with EBV [147].

Pathology

Morphology

The neoplasm often appears as multifocal ulcerating intestinal lesions consisting of a pleomorphic lymphoid infiltrate [1]. Jejunum and ileum are the most frequently affected sites. There is a predominance of atypical medium to large lymphoid cells with variable amount of pale cytoplasm, round or irregular vesicular nuclei, and prominent nucleoli. Anaplastic large cells or multinucleated giant cells may be present, mimicking ALCL. The infiltrate is commonly mixed with inflammatory cells such as histiocytes and eosinophils.

Immunophenotype

The neoplastic cells express CD3, CD7, TIA-1, granzyme B, and CD103. They are negative for CD4 and CD5. Tumors consisting of small- to medium-sized lymphoid cells may express CD8 and/or CD56 [1, 148]. In most cases, a various proportion of tumor cells are also CD30+ [148].

Molecular and Cytogenetic Studies

The *TCR* genes, most commonly γ and δ , are rearranged [149]. Loss of heterozygosity (LOH) at chromosome 9p21 and gains at chromosome 9q, 7q, 5q, and 1q have been reported in some cases [150–152].

Clinical Aspects

Most patients have a history of celiac disease and present with abdominal pain and sometimes evidence of intestinal perforation [1]. The clinical outcome is usually poor. Differential diagnosis includes inflammatory bowel disorders and other types of lymphomas.

ANAPLASTIC LARGE CELL LYMPHOMA

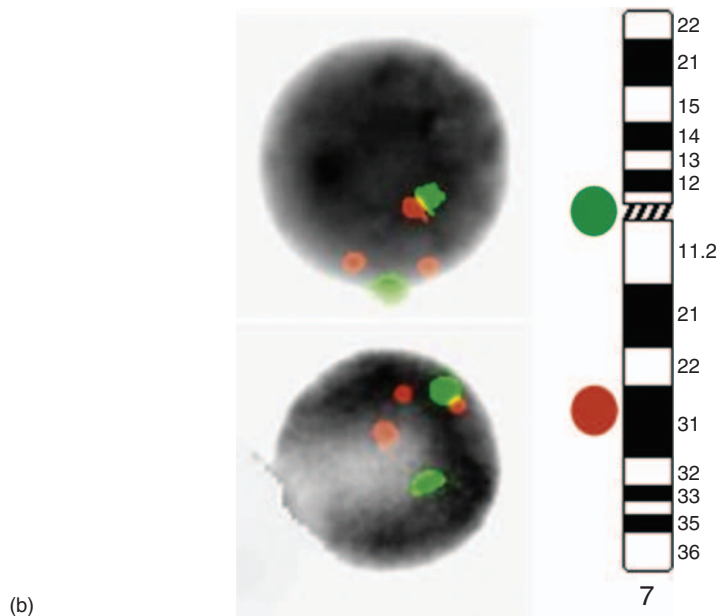
Anaplastic large cell lymphoma (ALCL) is a T-cell malignancy consisting of large anaplastic, pleomorphic CD30+ cells, often expressing ALK (large cell lymphoma kinase) and cytotoxic-associated proteins. The tumor cells have a tendency to grow cohesively in the lymph node sinuses, mimicking metastatic tumors [1, 153, 154].

Etiology and Pathogenesis

The etiology and pathogenesis of ALCL are not known. The strong association of ALCL with t(2;5)(p23;q35) suggests that this chromosomal translocation may play a role in the pathogenesis of this disease [154]. This translocation creates



FIGURE 17.35 Karyotype (a) and FISH (b) of tumor cells in a patient with hepatosplenic lymphoma demonstrating 46,XX,iso(7)(q10).



a hybrid gene as the result of the fusion of *NPM1* (nucleophosmin) gene on chromosome 5 with *ALK* gene on chromosome 2 [153, 156]. The NPM1-ALK protein activates the anti-apoptotic PI3K-Akt pathway and a number of signal transducers and activators of transcription proteins, which are all important in cellular transformation [157, 158]. The oncogenic properties of *NPM1-ALK* have been supported by *in vivo* studies in experimental animals [158, 159].

Pathology

Morphology

The affected lymph nodes are partially or totally effaced due to the infiltration of the tumor cells that often have a tendency to grow cohesively in the sinuses, resembling metastatic tumors [1, 153]. The tumor cells are usually admixed

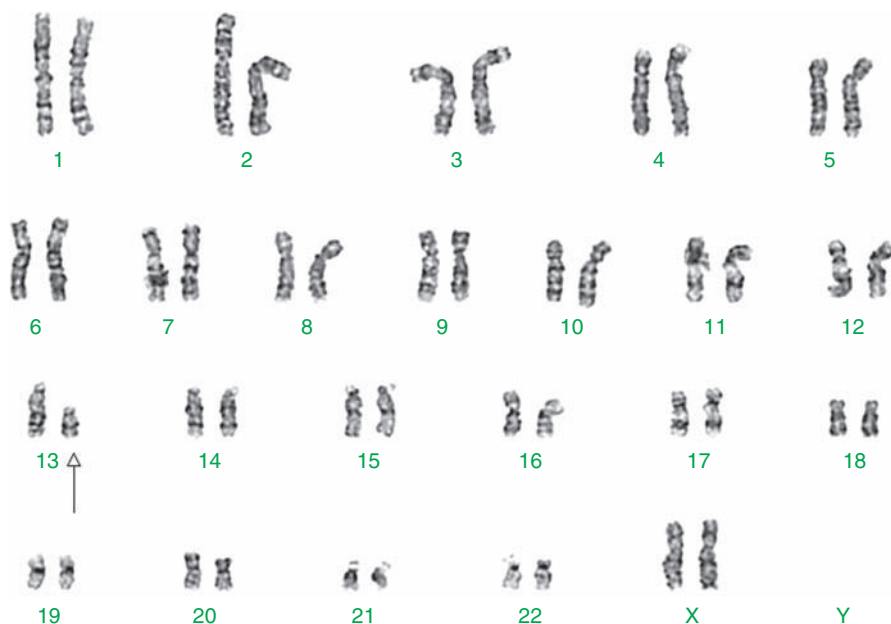


FIGURE 17.36 Karyotype of tumor cells in a patient with hepatosplenic lymphoma demonstrating 46,XX,del(13)(q12q22).

with inflammatory cells, which predominantly consist of histiocytes and plasma cells. Sclerotic thick capsule and well-formed fibrous bands are infrequent. Three major morphologic variants have been described: (1) common, (2) small cell, and (3) lymphohistiocytic variants [1, 153].

The *common variant* (70% of cases) is characterized by the predominance of large pleomorphic cells with abundant clear to light blue cytoplasm; often eccentric, horse-shoe, kidney-shaped, or multilobulated nuclei; and multiple small nucleoli (Figures 17.37 and 17.38). The large cells which have a horse-shoe or kidney-shaped nucleus next to the Golgi area are referred to as the “hallmark cells,” because they are detected in all morphologic types [1, 153]. The neoplastic cells may demonstrate cytoplasmic vacuoles in touch preparations. The multilobulated cells may show pseudonuclear inclusions (doughnut cells) due to the invagination of the nuclear membrane [1, 160]. Reed–Sternberg-like multinucleated cells may be present.

The *small cell variant* (5–10% of cases) consists of a mixture of large-, medium-, and small-sized pleomorphic cells. Small- and medium-sized cells are the predominant cells depicting a clear cytoplasm and an irregular nucleus [160]. The large neoplastic cells tend to cluster around small vessels.

The *lymphohistiocytic variant* (5–10% of cases) consists of small and large neoplastic cells including “hallmark” cells as well as large numbers of reactive histiocytes. Hemophagocytic macrophages may be present. The abundance of histiocytes may mask the neoplastic cell population in the H&E stains, but immunohistochemical stains for CD30 and ALK help to identify the tumor cells.

Other morphologic variants such as sarcomatoid form with large, bizarre spindle-shaped tumor cells and giant cell-rich type with numerous multinucleated giant cells have been reported [153, 160].

Immunophenotype

The large neoplastic cells characteristically express CD30 which is confined to the cell membrane and the Golgi

region. In the small cell variant, only the large anaplastic cells show strong positivity for CD30, whereas the small ones are either negative or weakly positive [160]. However, CD30 is not ALCL specific and is demonstrated on activated lymphocytes, and other types of B- and T-cell lymphoid malignancies [1, 153]. The tumor cells in the majority of the ALCL cases express epithelial membrane antigen (EMA), TCR, TIA-1, granzyme, and one or more T-cell-associated markers, particularly CD2 and CD4. The expression of CD3, CD5, and CD7 is less frequent and CD8 is usually negative [1]. Some ALCL cases may lack expression of T-cell-associated markers (previously considered “null” ALCL) [154]. CD43 and clusterin are expressed in the majority of the cases [161, 162]. Clusterin is a disulfide-linked heterodimeric protein associated with apoptosis. CD15 and EBV are usually negative in ALCL.

ALK expression is the most specific marker for the diagnosis of ALCL and is detected in 60–85% cases (Figure 17.39) [1]. ALK expression is cytoplasmic or nuclear, or both. Bcl-2 expression has been reported only in ALK-negative ALCL, whereas c-MYC nuclear expression is seen in ALK-positive pediatric cases but not ALK-negative ones [163].

Molecular and Cytogenetic Studies

The majority of the cases of ALCL demonstrate clonal *TCR* rearrangements and *NPM1-ALK* fusion gene [153, 156]. EBV sequences are not detected. The *NPM1-ALK* fusion is associated with a balanced $t(2;5)(p32;q35)$ (Figure 17.40) [158]. This translocation is the most common ALK-related chromosomal aberration, accounting for about 75% of all ALK-positive cases of ALCL. It is most readily and sensitively detected by FISH, but PCR-based molecular testing, similar to that used to detect the *BCR-ABL* fusion in chronic myeloid leukemia (CML), is available in some centers [164–167]. The remaining 25% show various ALK-related rearrangements including $t(1;2)(q21;p23)[TPM3-ALK]$, $t(2;3)(p23;q21)[ALK-TFG]$

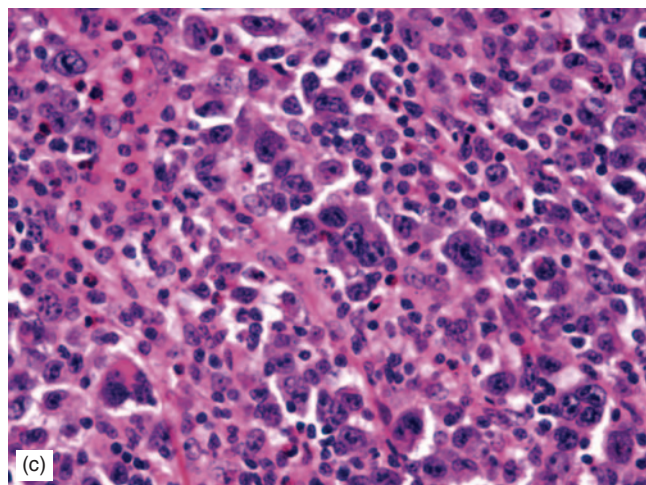
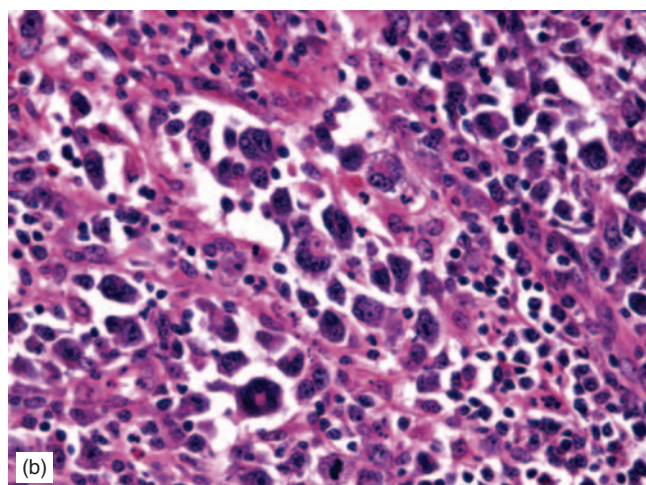
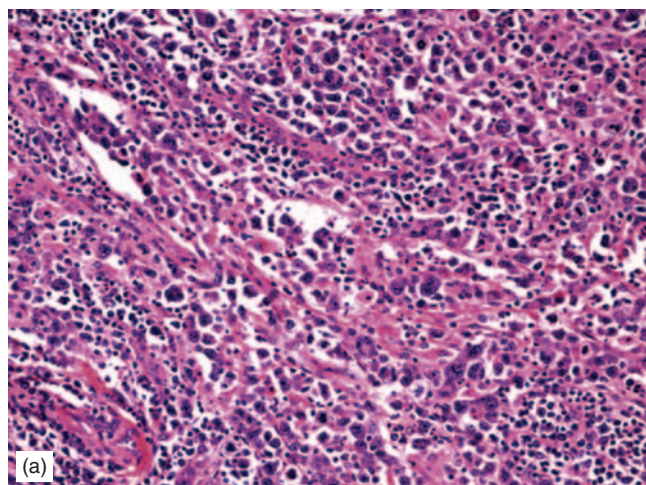


FIGURE 17.37 A lymph node section demonstrating ALCL with sinusoidal involvement. Horse-shoe and Hodgkin-like cells are present: (a) low power, (b) intermediate power, and (c) high power views.

(Figure 17.41), $t(2;17)(p23;q23)[ALK-CLTC]$, $t(X;2)(q11-12;p23)[MSN-ALK]$, and $inv(2)(p23q35)$ (Table 17.5) [156, 158, 168]. The expression of ALK in hematologic neoplasms is largely limited to ALCL tumors of T- or null-cell

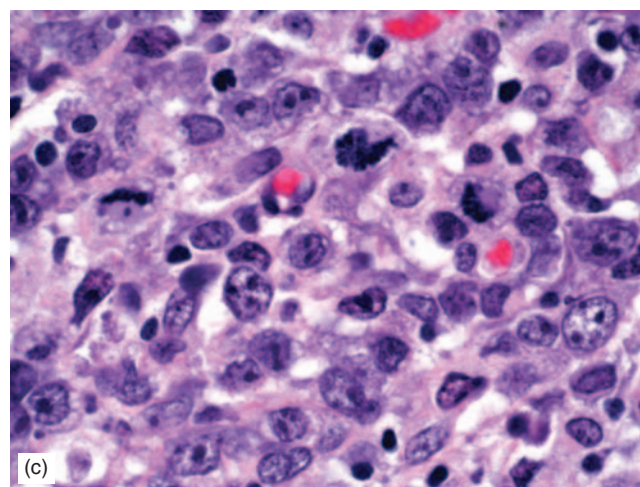
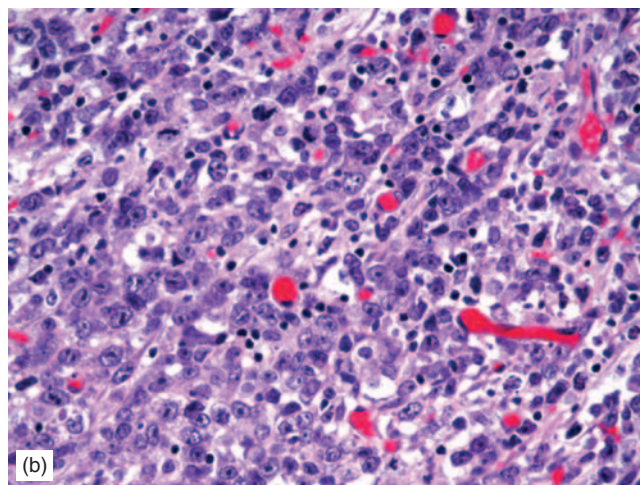
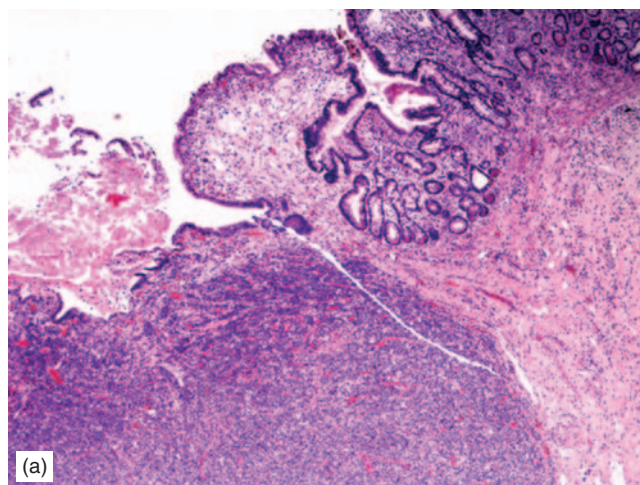


FIGURE 17.38 Gastric involvement with ALCL: (a) low power, (b) intermediate power, and (c) high power views.

immunophenotype [169]. Because multiple chromosomal regions are involved in the translocations with the 2p23 (*ALK* locus), the rearrangements can be easily identified by a 2p23-specific dual-color “breakapart” FISH probe.

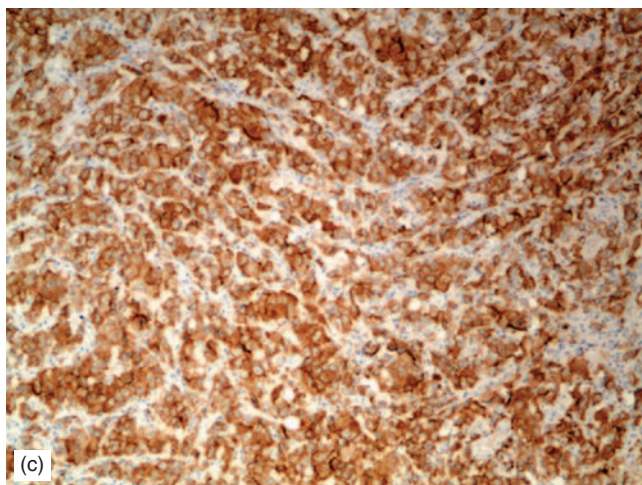
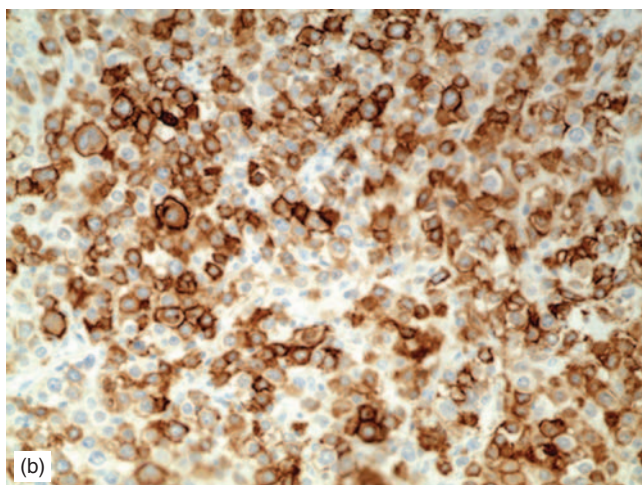
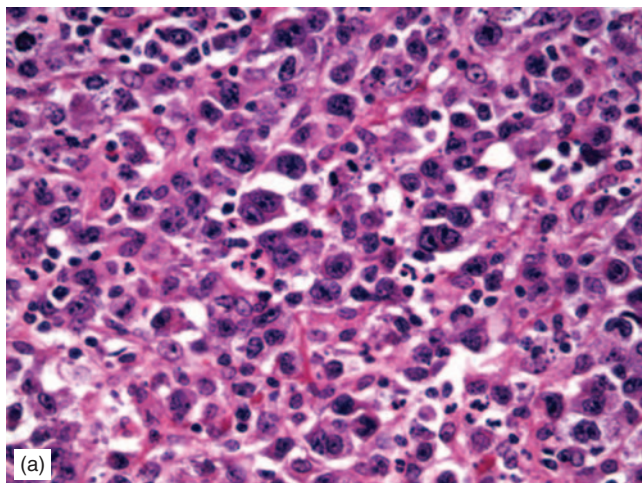


FIGURE 17.39 Anaplastic large cell lymphoma. H&E (a) and immunohistochemical stains demonstrating expression of CD30 (b) and ALK (c).

In many studies, evidence of the translocation has not only been observed in the patients, but also in the blood of healthy persons by RT-PCR studies [170].

The ALK overexpression is detected by immunohistochemical stains with anti-ALK antibodies. ALK-positive

staining in the classic t(2;5) is detected in nucleolus, nucleus, and cytoplasm; whereas other translocations lead to only cytoplasmic staining [1].

Recent studies of gene expression profiling of systemic ALCL have revealed differences between the ALK-positive and the ALK-negative subgroups. The ALK-positive tumors showed overexpression of *BCL6*, *PTPN12*, *CEBPB*, and *SERPINA1* genes; whereas *CCR7*, *CNTFR*, *IL22*, and *IL21* genes were overexpressed in the ALK-negative group [171]. Study of the gene expression profiles of four ALCL and three HD cell lines showed higher levels of *BCL3* expression in ALCL than HD cell lines. *BCL3* encodes a nuclear protein which belongs to the I κ B family of inhibitors of nuclear factor- κ B (NF- κ B) transcriptional factors [172]. Also, *JAK3* activation is significantly associated with ALK expression in ALCL [173].

Clinical Aspects

Primary systemic ALCL represents about 20–30% of the large cell lymphomas in children and 5% of all non-Hodgkin lymphomas in adults [153, 154, 160]. The male:female ratio is about 6:1 [153, 154]. Approximately 70% of the patients present with constitutional symptoms (mostly high fever and weight loss) and are in stage III/IV. Extranodal involvement is noted in about 60% of the patients, with skin (21%), bone (17%), and soft tissues (17%) being the most frequent sites [174, 175]. Approximately 10% of the patients may show liver or pulmonary involvement [153]. Leukemic presentation is uncommon.

ALCL is divided into two major groups: ALK-positive and ALK-negative. Several studies of a large series of ALCL patients have confirmed a significant prognostic difference between these two groups. The 5-year overall survival ranges from 70% to 90% in patients with ALK-positive tumors compared to 15–37% in patients with ALK-negative tumors [176–179]. The vast majority of the ALCLs in children and young adults are ALK-positive by immunohistochemical stains and show translocation of *ALK* gene [177, 180, 181]. ALCL accounts for 10–15% of childhood non-Hodgkin lymphomas.

Other factors associated with poor prognosis include mediastinal involvement, involvement of spleen, lung, or liver, CD56 expression, and small cell variant [182, 183].

Multiagent chemotherapy and autologous or allogeneic bone marrow transplantation are among the routine therapeutic approaches [184]. Anti-CD30 therapy and vaccination against ALK protein are under investigation [185].

Differential Diagnosis

ALCL may share some morphologic features with classical Hodgkin lymphoma. Sclerotic thick capsule and well-formed fibrous bands which are frequently seen in Hodgkin lymphoma are infrequent in ALCL. The Reed–Sternberg cells are CD45–, CD15+, CD30+, EMA–, and ALK–, and may express CD20 and show EBV positivity; whereas the neoplastic cells of ALCL are CD45+, CD15–, ALK±, EMA+, may express some of pan-T-cell markers, and may be positive or negative for EBV. Sinusoidal involvements in

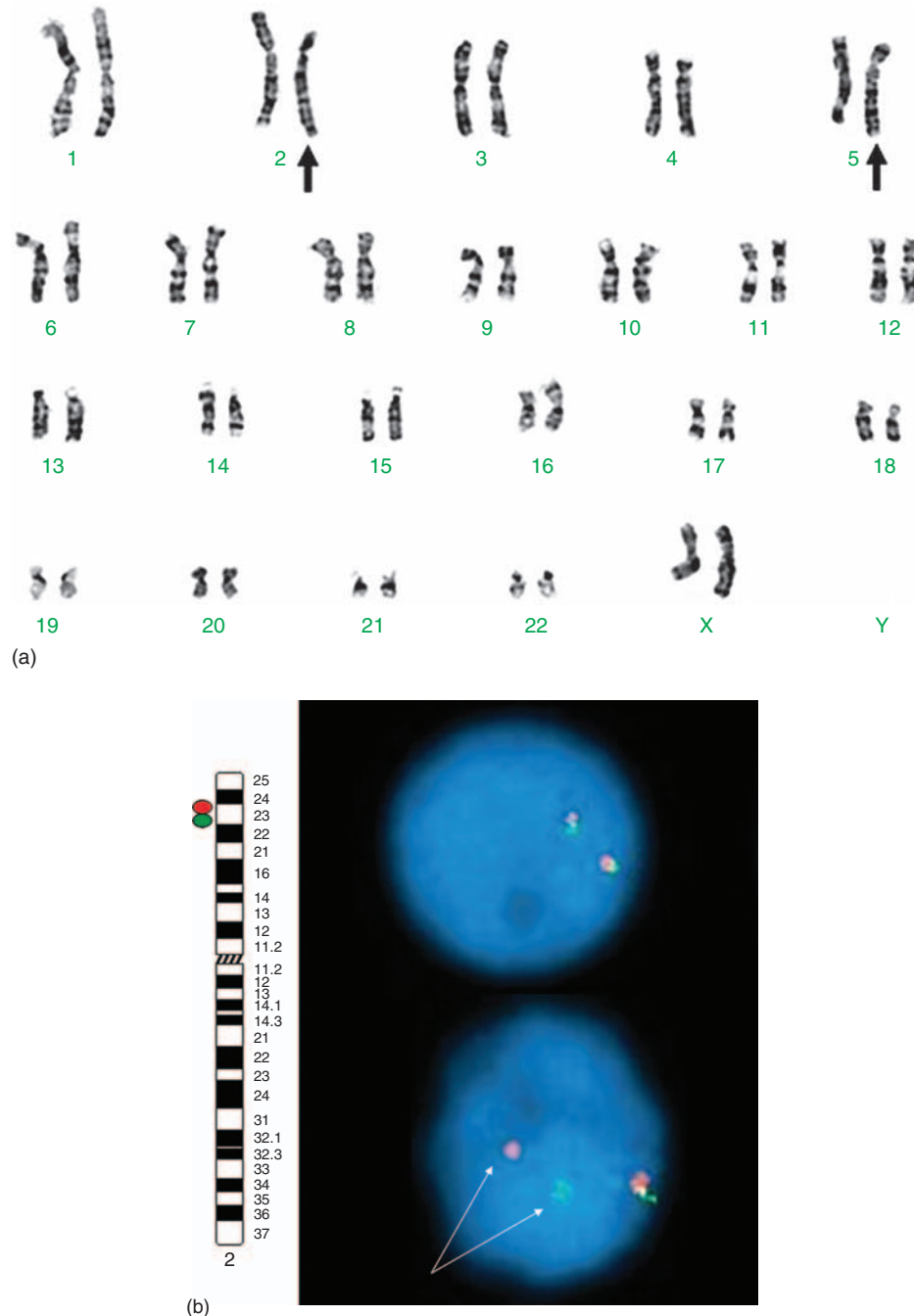


FIGURE 17.40 (a) Karyotype of tumor cells of a patient with ALCL demonstrating $t(2;5)(p23;q35)$. (b) FISH with ALK(2p23) dual-color, breakapart rearrangement probe showing split signals.

the lymph nodes and bone marrow may mimic metastatic carcinoma.

PRIMARY CUTANEOUS ALCL

Primary cutaneous ALCL is a CD30+, ALK– lymphoma of anaplastic large cells confined to the skin, most often presenting as a solitary lesion [111, 120, 186, 187].

Etiology and Pathogenesis

The etiology and pathogenesis of this disorder are not known.

Pathology

Morphology

The morphologic features are similar to the systemic ALCL. The upper and deep dermis is diffusely infiltrated

with anaplastic large cells, often with numerous Reed–Sternberg-like cells and/or multinucleated giant cells [111]. The epidermis may be infiltrated or ulcerated. A modest inflammatory background may be present.

Immunophenotype

The neoplastic cells are usually positive for CD4, CD30, TIA-1, and granzyme B. Approximately 50% of the cases are positive for HECA-452, which marks for the cutaneous lymphocyte antigen [111, 187]. Loss of expression of some of the pan-T-cell markers, such as CD2, CD3, or CD5, is frequent. Most cases of cutaneous ALCL are negative for ALK and EMA.

TABLE 17.5 Chromosomal aberrations in ALK-positive ALCL.*

Aberrations	Involved genes**	Frequency (%)
t(2;5)(p23;q35)	ALK/NPM	~75
t(1;2)(q21;p23)	TPM3/ALK	~15
t(2;3)(p23;q21)	ALK/TFG	~2
t(2;17)(p23;q23)	ALK/CLTC	~2
t(X;2)(q11-12;p23)	MSN/ALK	~1
inv(2)(p23;q35)	ALK/AT1C	~2

*Adapted from Ref. [158].

**ALK: anaplastic lymphoma kinase; CLTC: clathrin heavy chain; MSN: moesin; NPM: nucleophosmin; TFG: TRCK fusion gene; TPM: tropomyosin.

Molecular and Cytogenetic Studies

Most cases show *TCR* gene rearrangement [111] and are commonly negative for *NPM1-ALK* fusion gene or t(2;5) or other *AKL*-related translocations observed in the systemic ALCL.

Clinical Aspects

Primary cutaneous ALCL accounts for 10–25% of cutaneous lymphomas [1, 111]. It usually occurs in elderly patients with a median age of about 60 years. The male:female ratio is about 1.5–2:1 [1, 111]. It typically presents as a solitary, asymptomatic cutaneous reddish-violet tumor, sometimes superficially ulcerated. Multiple skin lesions are infrequent. Approximately 25% of the patients show partial or complete spontaneous regression. Disease-specific survival at 5 years for localized lesions is 91% compared to 50% for the generalized cutaneous ALCL [188]. The conventional treatment for localized lesions is excision with or without radiation. Combination chemotherapy is recommended for disseminated skin disease [153].

Differential Diagnosis

The differential diagnosis includes lymphomatoid papulosis, Hodgkin lymphoma, CD30+ large cell transformation of MF, secondary cutaneous involvement of ALCL, and CD30+ cutaneous NK/T-cell lymphoma [188].

Lymphomatoid papulosis is a benign, chronic recurrent skin disorder characterized by multiple spontaneously

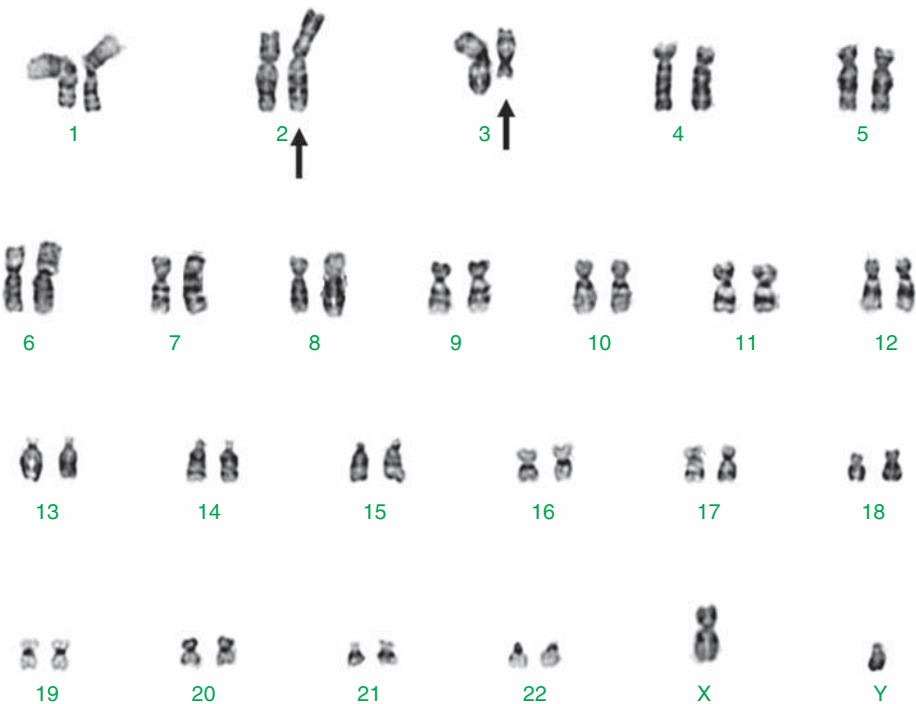


FIGURE 17.41 Karyotype of tumor cells of a patient with ALCL demonstrating 46,XY,t(2;3)(p23;q21).

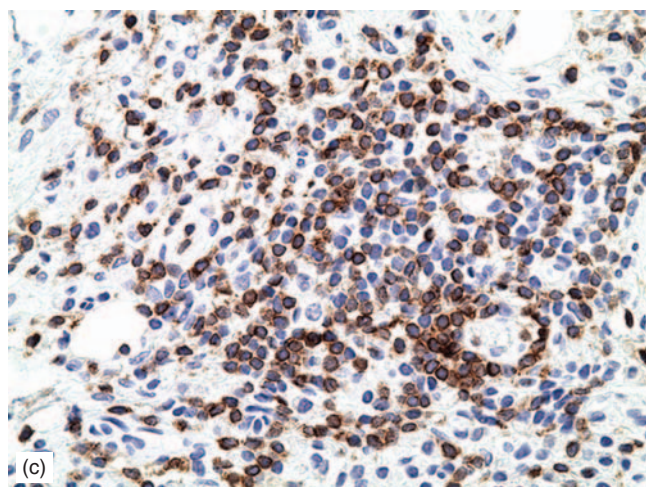
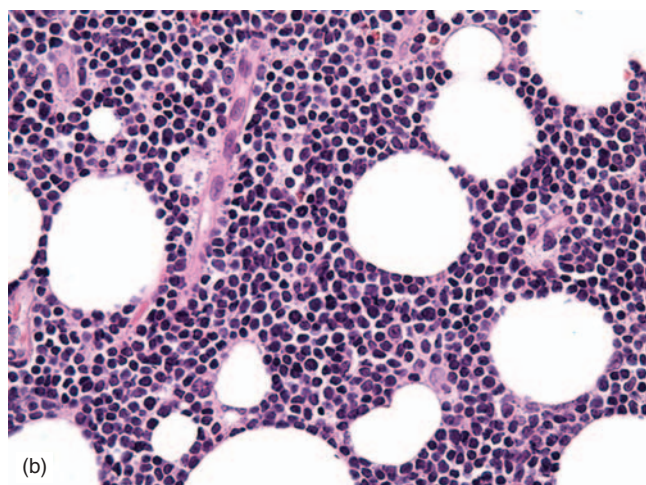
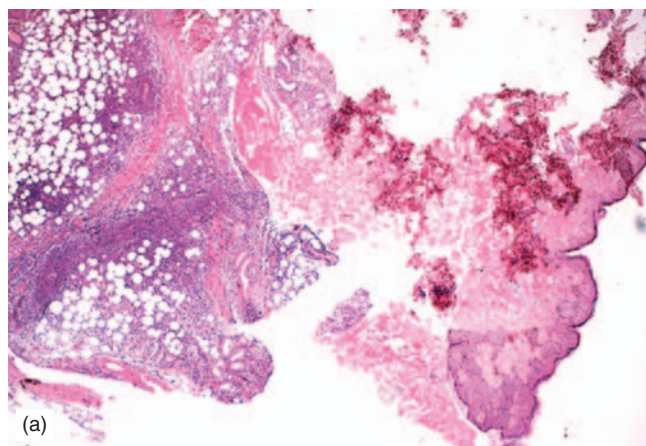


FIGURE 17.42 Subcutaneous panniculitis-like T-cell lymphoma: (a) low power and (b) high power views. (c) The infiltrating lymphocytes are CD3+.

regressing papules consisting of a polymorphic infiltrate including anaplastic large lymphocytes and cells resembling Reed–Sternberg cells. These cells represent activated CD4+ cells and coexpress CD30, but are negative for ALK (see Chapter 19).

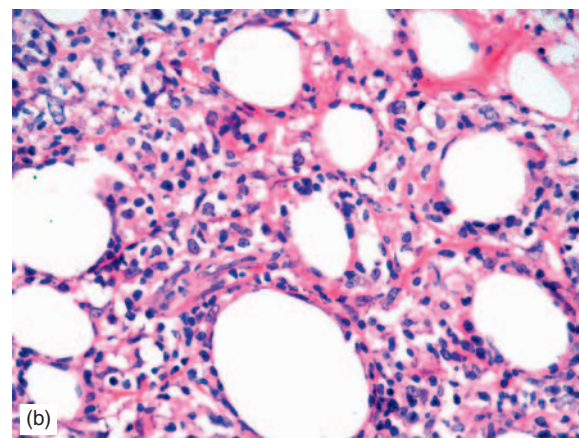
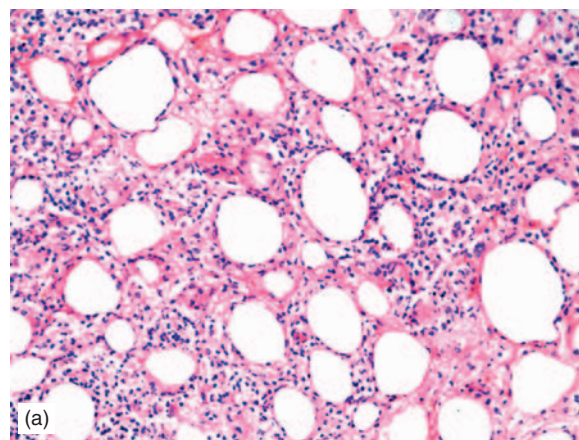


FIGURE 17.43 Subcutaneous panniculitis-like T-cell lymphoma infiltrating subcutaneous fatty tissue: (a) low power and (b) high power views.

OTHER PRIMARY CUTANEOUS T-CELL LYMPHOMAS

The classification recently proposed by the World Health Organization and European Organization for Research and Treatment of Cancer (WHO/EROTC) divides the previously known *subcutaneous panniculitis-like T-cell lymphoma* into two separate categories: (1) subcutaneous panniculitis-like T-cell lymphoma and (2) cutaneous $\gamma\delta$ T-cell lymphoma [145]. These two entities and primary cutaneous aggressive epidermotropic CD8+ cytotoxic T-cell lymphoma are briefly discussed in this section.

Subcutaneous Panniculitis-Like T-Cell Lymphoma

Subcutaneous panniculitis-like T-cell lymphoma is a rare lymphoma which involves subcutaneous fat without dermal or epidermal infiltration, leading to erythematous or violaceous nodules, plaques, or both [189, 190]. The infiltrate consists of a mixture of small and medium to large atypical cells with areas of necrosis (Figures 17.42 and 17.43).

The neoplastic T-cells have a tendency to rim around adipocytes. The infiltrate often contains reactive histiocytes, which may show hemophagocytosis [189]. The neoplastic cells represent TCR $\alpha\beta$ class and often express CD3, CD8, granzyme B, and TIA-1. The tumor cells in most patients show clonal *TCR* gene rearrangement [189]. The EBV association is controversial [190–192].

Subcutaneous panniculitis-like T-cell lymphoma is often associated with a systemic hemophagocytosis characterized by fever, hepatosplenomegaly, lung infiltrates, liver dysfunction, coagulation abnormalities, and pancytopenia [189]. This hemophagocytic syndrome may develop before or during the manifestation of T-cell lymphoma [191].

The clinical manifestation of subcutaneous panniculitis-like T-cell lymphoma is variable, ranging from indolent course to a rapidly fatal hemophagocytic process. Local radiation therapy and/or systemic chemotherapy are used. The 5-year survival rate has been reported as 80% in a recent study [193].

Cutaneous $\gamma\delta$ T-Cell Lymphoma

Cutaneous $\gamma\delta$ T-cell lymphoma was previously considered a subtype of subcutaneous panniculitis-like T-cell lymphoma, accounting for 25% of the cases. Morphologic features are similar to those of subcutaneous panniculitis-like, except that dermal and epidermal involvement may be present. Cutaneous $\gamma\delta$ T-cell lymphoma is a more aggressive disease than subcutaneous panniculitis-like T-cell lymphoma [189, 193]. The neoplastic cells are of TCR $\gamma\delta$ type, usually negative for CD4 and CD8 and positive for CD56 [1, 194]. Note that Southern blot analysis using the T $\alpha\beta$ probe will be negative in these cases, but PCR analysis for TCR γ will usually be informative.

Primary Cutaneous Aggressive Epidermotropic CD8+ Cytotoxic T-Cell Lymphoma

Primary cutaneous aggressive epidermotropic CD8+ cytotoxic T-cell lymphoma is characterized by localized or disseminated eruptive skin lesions (papules, nodules and tumors) with epidermal infiltration of CD8+ cytotoxic T-cells and an aggressive clinical course [194].

ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA

Angioimmunoblastic T-cell lymphoma (AITL) is a peripheral T-cell lymphoma characterized by generalized lymphadenopathy, hepatosplenomegaly, anemia, hypergammaglobulinemia, and a polymorphic infiltrate involving germinal center T-helper (GC-Th) cells and follicular dendritic cells [196, 197].

Etiology and Pathogenesis

The etiology and pathogenesis of this disorder are not clearly understood. Molecular and immunophenotypic studies

suggest that the neoplastic cells of AITL derive from GC-Th cells [196, 197]. These helper T-cells are positive for CD10 and Bcl-6 and show overexpression of CXCL13, a chemokine critical for lymphocyte entry into germinal centers [198]. There have been reports of association between AITL and a number of lymphotropic viruses, such as EBV, human herpes virus (HHV), HIV, and hepatitis C virus, but their role in the pathogenesis of AITL is controversial [199].

Pathology

Morphology

The involved lymph nodes display partial or total effacement of nodal architecture by a polymorphic infiltrate, predominantly involving interfollicular areas. The lymph node sinuses are usually well preserved. There is often pericapsular infiltration. Three overlapping morphologic patterns have been described [197, 200, 201].

Pattern I represents about 20% of the cases with preservation of nodal architecture. It is characterized by follicular hyperplasia, poorly developed mantle zones, and expanded paracortex with a polymorphic infiltrate consisting of lymphocytes, eosinophils, plasma cells, macrophages, transformed large lymphoid blasts, occasional Reed–Sternberg-like cells, and vascular proliferation with abundant endothelial venules.

Pattern II accounts for about 30% of the AITL cases. In Pattern II the normal architecture is almost completely lost, except for occasional depleted follicles showing concentrically arranged follicular dendritic cells. In some cases proliferation of the follicular dendritic cells may extend beyond the follicles. A polymorphic infiltrate with numerous transformed blast cells and vascular proliferation is present.

Pattern III represents about 50% of the cases and is characterized by complete effacement of nodal architecture, prominent proliferation of follicular dendritic cells, extensive vascular proliferation, and in some cases, perivascular collections of atypical medium- to large-sized lymphoid cells with clear or pale cytoplasm (Figure 17.44).

In some cases, consecutive biopsies from the same patient have shown a transition from patterns I to III, suggesting progression from an early stage to an advanced stage [197, 200, 201].

Bone marrow, spleen, liver, skin, and lungs are the most frequent extranodal sites of involvement (Figure 17.45). The involvement of the extranodal sites is often non-specific and consists of a polymorphic infiltrate mimicking an inflammatory process [197].

Immunophenotype

The immunophenotypic hallmark of AITL is the presence of CD4+ T-cells coexpressing CD10 and Bcl-6 within the polymorphic lymphoid infiltrate along with increased numbers of follicular dendritic cells expressing CD21, CD23, or CD35 (Figures 17.46 and 17.47) [197, 200–205]. The CD4+ cells are admixed with CD8+ cells, plasma cells

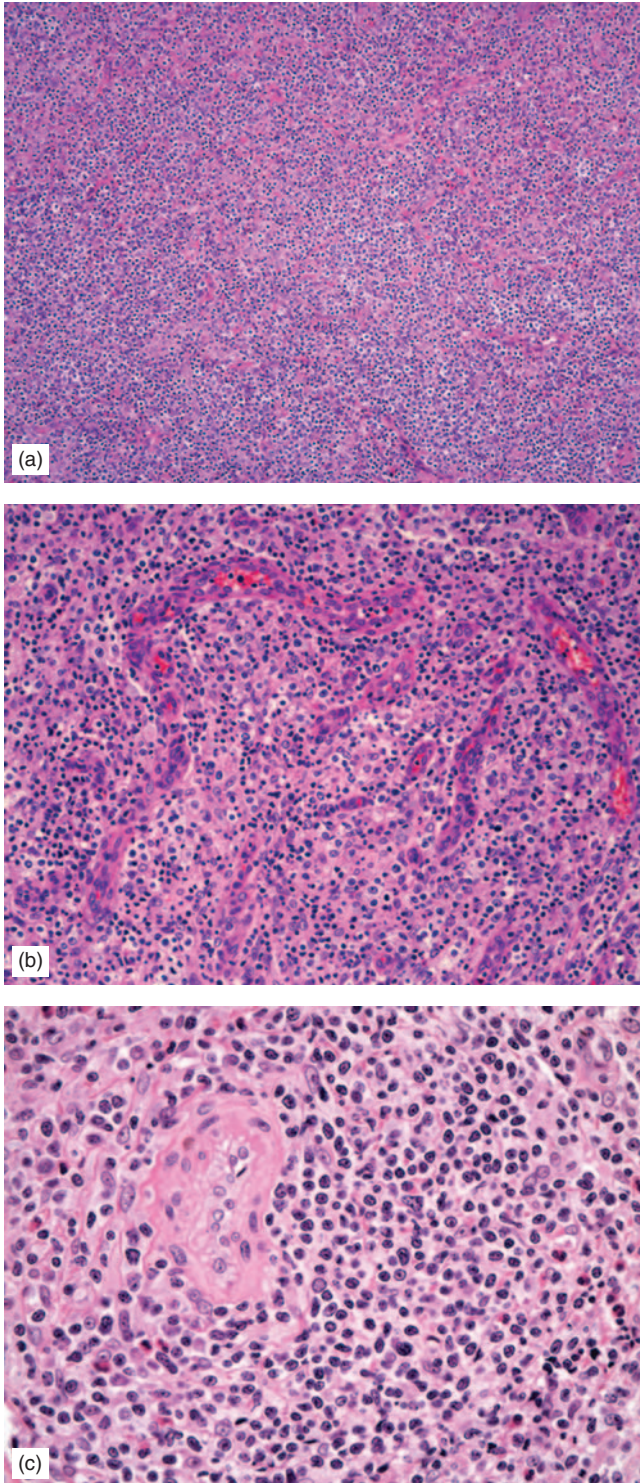


FIGURE 17.44 Angioimmunoblastic T-cell lymphoma demonstrating vascular proliferation and predominance of atypical medium- to large-sized lymphoid cells with clear or pale cytoplasm: (a) low power, (b) intermediate power, and (c) high power views.

(CD138+), and histiocytes (CD68+). The residual follicles are identified by CD20 and CD79a. The CD4+/CD10+ (GC-Th) cells and follicular dendritic cells may be more prominent around high endothelial venules [206, 207].

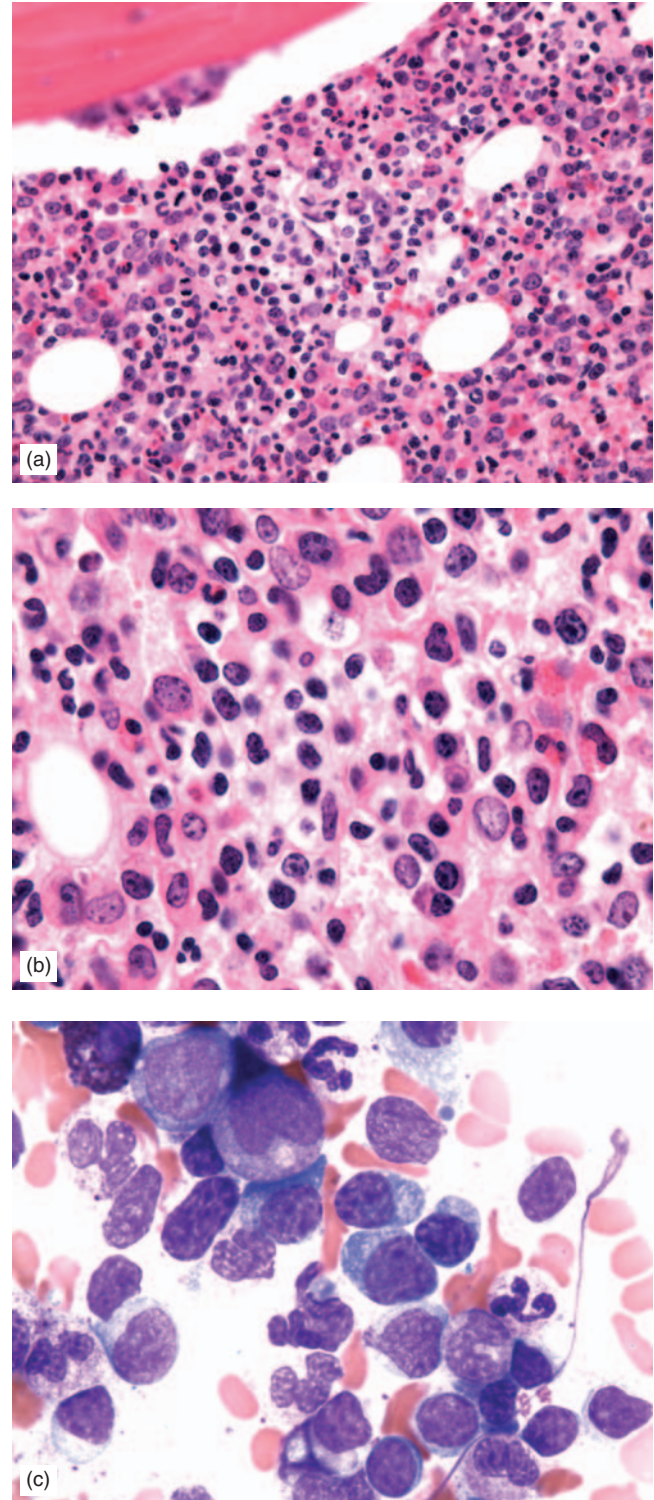


FIGURE 17.45 Bone marrow involvement in angioimmunoblastic T-cell lymphoma may appear non-specific with a collection of histiocytes and atypical lymphocytes: (a) low power and (b) high power views of biopsy section, and (c) bone marrow smear.

Molecular and Cytogenetic Studies

The *TCR* rearrangement studies reveal clonal rearrangement in over 75% of the cases [200, 207]. The affected

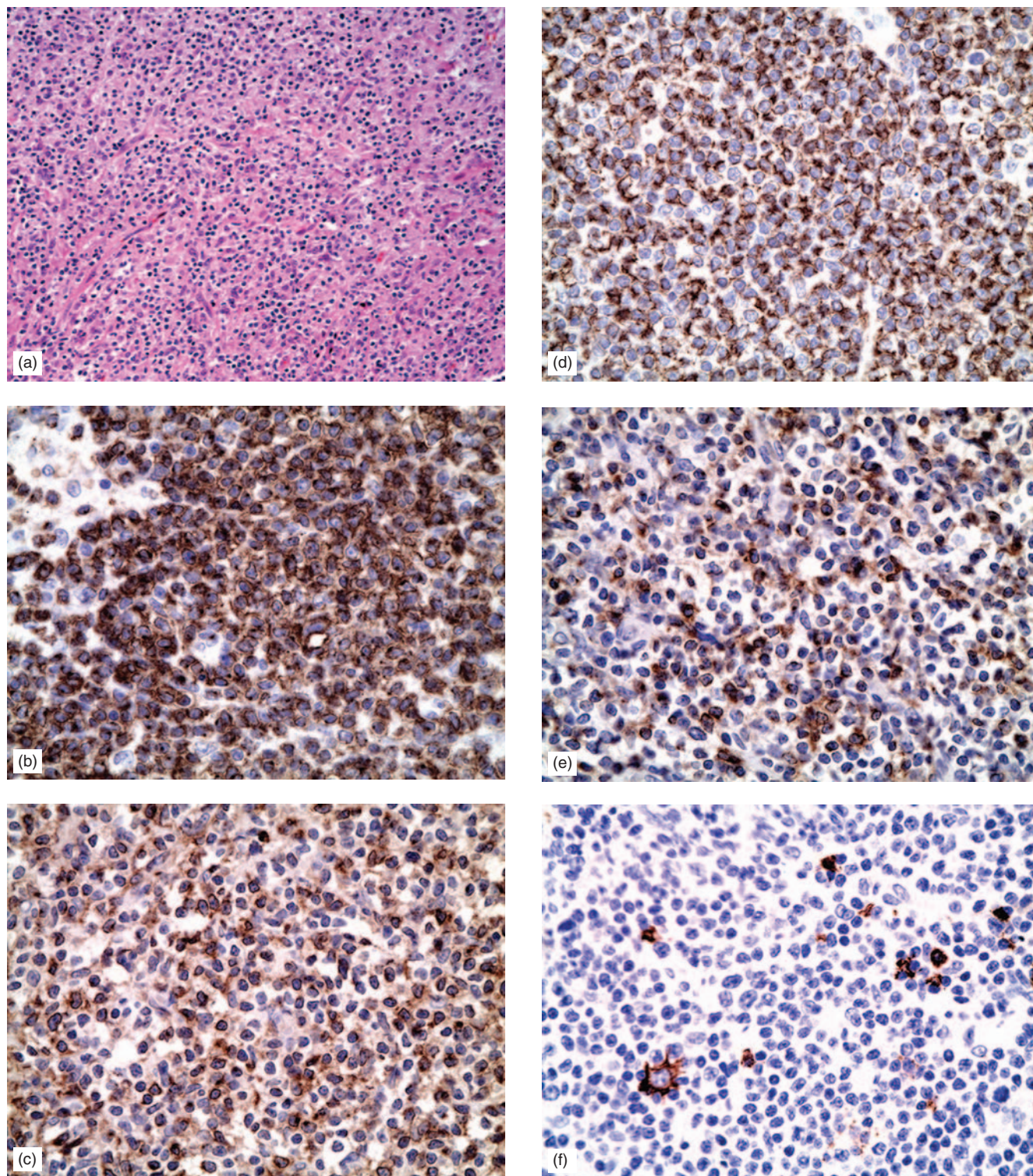


FIGURE 17.46 Angioimmunoblastic T-cell lymphoma. H&E (a) and immunohistochemical stains demonstrating expression of CD2 (b), CD3 (c) CD4 (d), CD10 (e), and CD20 (f).

lymph nodes in approximately 10% of the cases may show an expanded monoclonal B-cell population, often in association with increased number of EBV-infected large B-cells [197].

The gene expression profile of AITL cases has revealed overexpression of several gene characteristics of

GC-Th cells, such as *CXCL13*, *BCL6*, *PDCD1*, *CD40L*, and *NFATC1* [208].

Approximately 70% of the AITL patients show clonal chromosomal aberrations [196, 197, 209]. The most frequent recurrent abnormalities include trisomy 3, trisomy 5, and an additional X chromosome (Table 17.6). Other

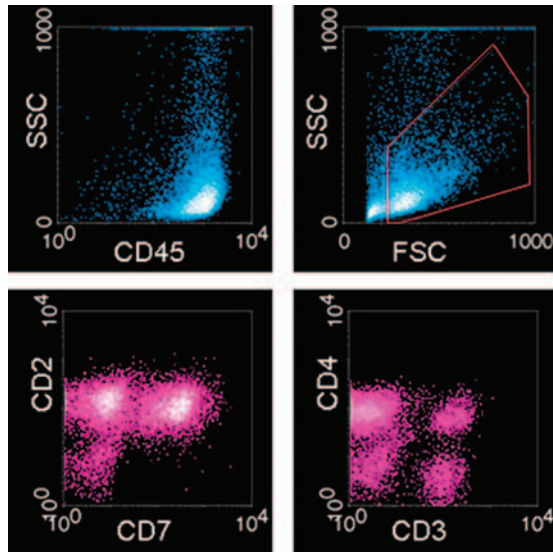


FIGURE 17.47 Angioimmunoblastic T-cell lymphoma. Flow cytometry demonstrating a large cell population (gated red) primarily consisting of CD2+, CD4+, and CD7+ cells.

TABLE 17.6 Clinical features and laboratory findings in angioimmunoblastic T-cell lymphoma.*

Symptoms and signs	Frequency (%)
B symptoms	68–85
Generalized lymphadenopathy	94–97
Splenomegaly	70–73
Hepatomegaly	52–72
Skin rash	48–58
Effusions	23–37
<i>Laboratory findings</i>	
Anemia	40–57
Hypergammaglobulinemia	50–83
Autoantibodies	66–77
Elevated LDH	70–74
Bone marrow involvement	61
Clonal cytogenetic aberrations such as +3, +5, and +X	70
TCR rearrangement	~100

*Adapted from Ref. [197].

frequently observed recurrent cytogenetic abnormalities are gains of 11q13, 19, and 22q [210].

Clinical Aspects

AITL accounts for about 2% of all non-Hodgkin lymphomas. The peak incidence is between the sixth and seventh

decades with no significant sex predilection and wide geographical distribution [197, 211]. The clinical presentation often mimics an infectious process characterized by B symptoms and generalized lymphadenopathy [200, 212]. Hepatosplenomegaly has been reported in 50–70% of the patients. Around 50% of the patients complain of pruritus and/or show skin rashes (Table 17.6). A garden variety of autoimmune disorders have been observed in association with AITL, such as autoimmune hemolytic anemia, polyarthrititis, rheumatoid arthritis, autoimmune thyroiditis, and vasculitis [212, 213]. Laboratory findings include anemia or pancytopenia, hypergammaglobulinemia, circulating autoantibodies, and elevated serum LDH (Table 17.6).

Single agent chemotherapy (steroids or methotrexate) and various combination of chemotherapeutic regimens have been tried with an overall discouraging outcome and a 5-year survival rate of 30–35% [197, 212].

Differential Diagnosis

The differential diagnosis includes various viral infections and collagen vascular disorders. Bone marrow biopsy and fine needle aspiration or needle core biopsy of the enlarged lymph node usually do not yield a definitive diagnosis. Diagnosis is achieved by the morphologic examination of the entire lymph node [197].

PERIPHERAL T-CELL LYMPHOMA, UNSPECIFIED

Peripheral T-cell lymphoma, unspecified (PTCL-u), includes all the T-cell lymphomas that are not included in the well-defined clinicopathologic entities previously described in this chapter. PTCL-u is predominantly nodal and represents the most common T-cell lymphomas in the Western countries [145, 211, 214].

Etiology and Pathogenesis

The etiology and pathogenesis of this heterogeneous group of neoplastic disorders are not known.

Pathology

Morphology

The normal nodal architecture is often effaced with a diffuse infiltration of neoplastic lymphoid cells. A garden variety of morphologic features have been described, but in most cases the predominant cells are medium- to large-sized with irregular nuclei. The nuclei are hyperchromatic or vesicular with prominent nucleoli. Mitotic figures are frequent. Large cells with clear cytoplasm and Reed–Sternberg-like cells may be present. Vascular proliferation is frequently noted [1]. The infiltrating neoplastic cells are often mixed with inflammatory cells such as small lymphocytes, eosinophils, and histiocytes. Histiocytes may appear in aggregates.

Two major subtypes have been described in the WHO classification: (1) T-zone and (2) lymphoepithelioid cell variants [1].

The *T-zone variant* is characterized by the expansion of interfollicular spaces as the result of infiltration of predominantly small- to medium-sized lymphocytes (Figure 17.48). Lymphoid follicles are usually well preserved or even hyperplastic [1]. Clusters of lymphoid cells with clear cytoplasm are often present, and scattered Reed–Sternberg-like cells may be present. There is a vascular proliferation with predominance of high endothelial venules. Inflammatory cells, such as eosinophils, plasma cells, and histiocytes, are commonly present.

The *lymphoepithelioid cell variant* (Lennert lymphoma) is characterized by the presence of numerous small aggregates of epithelioid histiocytes mixed with a lymphocytic infiltrate predominantly consisting of small lymphocytes with slightly irregular nuclei (Figure 17.49). Scattered larger lymphocytes with clear cytoplasm may be present. The pattern of lymphoid infiltration is diffuse but less frequently may be interfollicular. Vascular proliferation and presence of inflammatory cells are common features.

In a large multicenter retrospective analysis of 385 patients with PTCL-u, cases were divided into a number of morphologic subtypes such as large cell, large- and medium-sized cell, pleomorphic cell, small cell, lymphoepithelioid, T-zone, and PTCL-u not otherwise specified (Table 17.7). The most frequent morphologic subtype in this report was the large cell type accounting for 42% of the cases. Lymphoepithelioid and T-zone types presented 4.9% and 3.8% of the cases, respectively.

Immunophenotype

The neoplastic cells usually express pan-T-cell markers, such as CD3, CD5, and CD7, but loss of CD5 or CD7 is not infrequent. The tumor cells are more commonly CD4+ than CD8. Scattered cells may express CD30 [1, 145, 215]. Expression of CD56 or cytotoxic-associated proteins is rare, and EBV is usually negative in the tumor cells [1, 216].

Molecular and Cytogenetic Studies

Approximately 90% of the patients with PTCL-u show clonally rearranged *TCR* genes and 70–90% demonstrate cytogenetic aberrations [145]. A novel $t(5;9)(q33;q22)$ has been reported in 17% of PTCL-u [217]. This translocation fuses *ITK* and *SYK* genes together. Complex karyotype consistent with clonal evolution is a frequent finding. Breaks involving the *TCR* loci are often seen.

The chromosomes most frequently altered in structural aberrations are 1, 6, 2, 4, 11, 14, and 17. Additionally, trisomies of 3 or 5 and an extra X chromosome are also common [218, 219]. CGH studies found recurrent losses on chromosomes 13q21, 6q21, 9p21, 10q23–24, 12q21–22, and 5q (Figure 17.50) [220]. Recurrent gains were found on chromosome 7q22. High-level amplifications of 12p13 were observed in a few PTCL-u cases with cytotoxic phenotype. These results suggest that certain genetic alterations may indeed exist in PTCL-u, but definitive

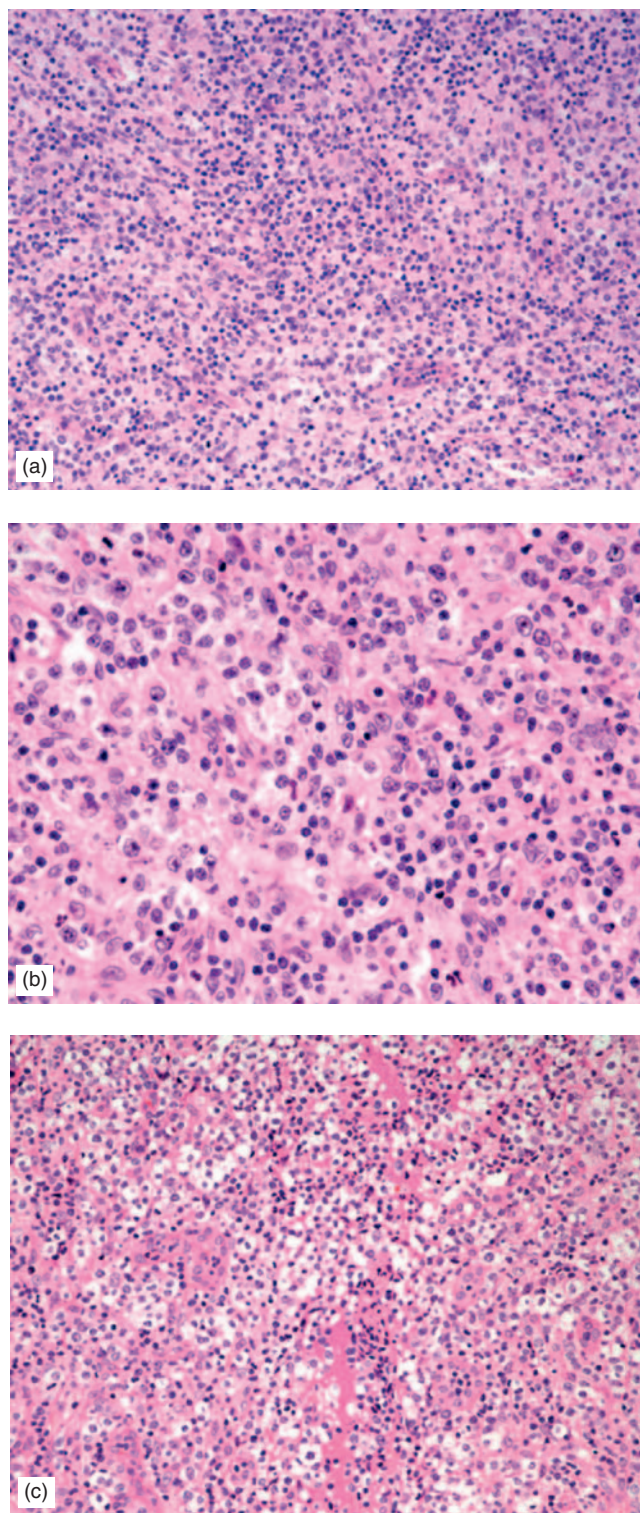


FIGURE 17.48 Peripheral T-cell lymphoma; the T-zone variant consisting of a mixture of small to large lymphocytes (a and b) and the presence of lymphoid cells with clear cytoplasm (c).

clinicopathologic subgroups have not yet been identified, and, thus far, various molecular and cytogenetic findings do not allow a consistent model for pathogenesis to be constructed.

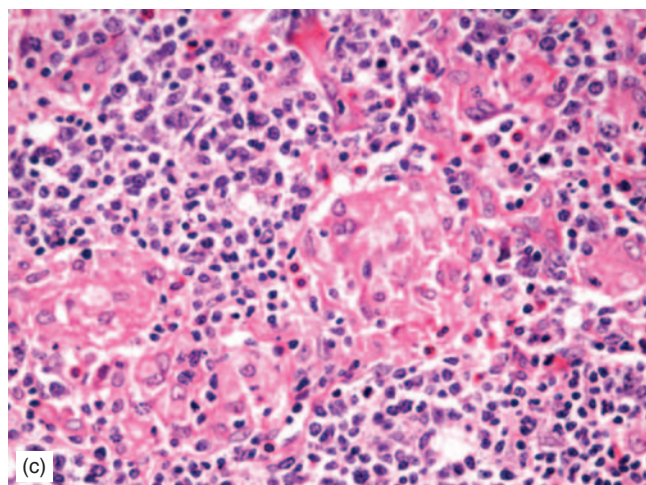
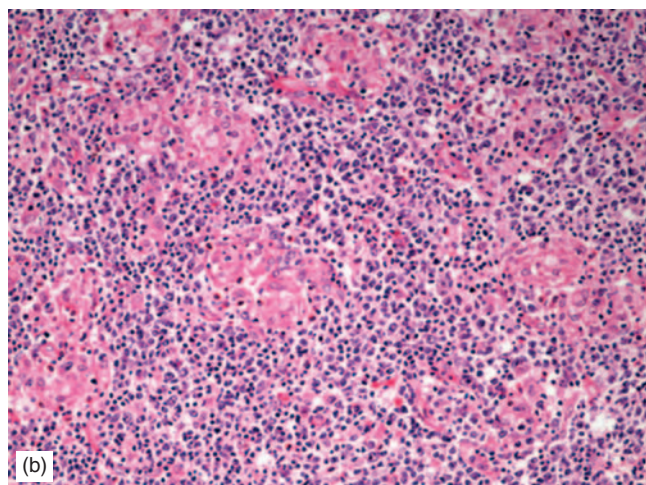
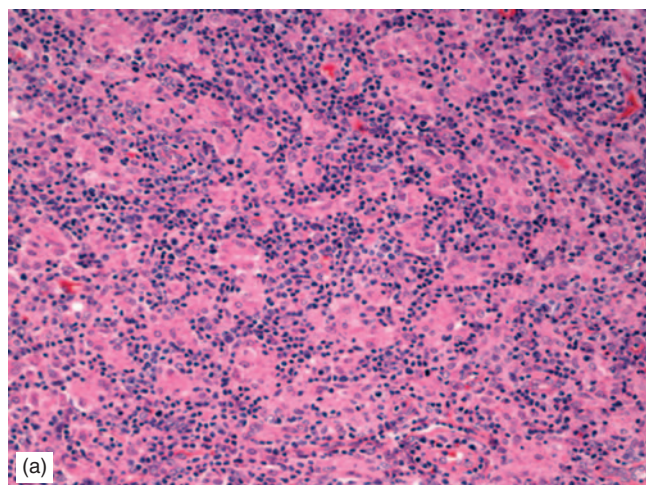


FIGURE 17.49 Peripheral T-cell lymphoma; the lymphoepithelioid cell variant (Lennert lymphoma). Epithelioid histiocytes are mixed with infiltrating lymphocytes in small (a) or large (b and c) clusters.

Clinical Aspects

PTCL-u is the most frequent peripheral T-cell lymphoma comprising approximately 60–70% of all T-cell lymphomas

TABLE 17.7 Morphologic subtypes observed in the retrospective study of 385 patients with peripheral T-cell lymphoma, unspecified.*

Morphologic subtype	% cases
T-zone	3.8
Lymphoepithelioid	4.9
Small cell	5.9
Pleomorphic cell	8.0
Large- and medium-sized cell	16.6
Large cell	18.4
PTCL-u not otherwise specified	42.0

*Adapted from Ref. [221].

[211]. It occurs in middle-aged to elderly patients with a median age of 54 years and a male:female ratio of about 1–2:1 [221]. About 65–75% of the patients are in advanced stages (stage III/IV) at diagnosis [145, 221]. Extranodal disease, B symptoms, and elevated LDH are frequent findings and are associated with unfavorable prognosis. The most frequently involved extranodal sites are bone marrow, spleen, liver, Waldeyer ring, and skin (Table 17.8).

In a large multicenter retrospective analysis of 385 patients with PTCL-u, multivariate analysis revealed four risk factors: (1) age >60 years, (2) LDH values at normal levels or above, (3) performance status (PS) ≥ 2 , and (4) bone marrow involvement [221]. The following four prognostic groups were identified:

Group 1 with no risk factors and 5-year survival rate of 62%

Group 2 with one risk factor and 5-year survival rate of 53%

Group 3 with two risk factors and 5-year survival rate of 33%

Group 4 with three or four risk factors and 5-year survival rate of 18%.

Molecular and immunophenotypic studies have demonstrated that *p53* mutation and overexpression of *p53* protein correlate with treatment failure and unfavorable prognosis [145, 222, 223].

Differential Diagnosis

The differential diagnosis includes various reactive lymphadenopathies, ALCL, AITL, and Hodgkin lymphoma. The interfollicular expansion and the presence of inflammatory cells in the T-zone variant may mimic T-zone hyperplasia. Presence of clusters of epithelioid histiocytes in the lymphoepithelioid cell (Lennert) variant may simulate

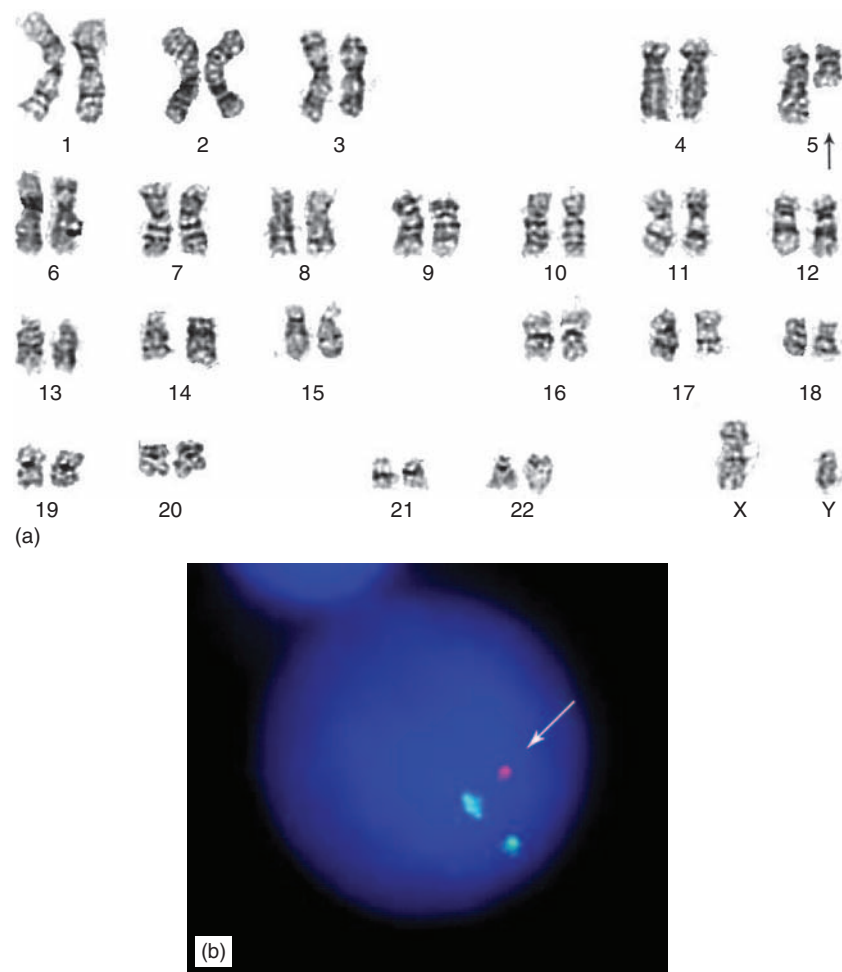


FIGURE 17.50 Deletion of 5q in a patient with peripheral T-cell lymphoma: (a) karyotype and (b) FISH analysis.

TABLE 17.8 Extranodal involvement in patients with peripheral T-cell lymphoma, unspecified.*

Site	% cases
Bone marrow	30.6
Spleen	24.6
Liver	12.9
Waldeyer ring	10.9
Skin	10.1
Lung and pleura	9.8
Bone	4.6
Soft tissue	1.2

*Adapted from Ref. [221].

toxoplasmosis, sarcoidosis, or other types of granulomatous lymphadenitis. Cases with increased proportion of anaplastic large cells or the presence of Reed–Sternberg-like cells may mimic ALCL or Hodgkin lymphoma, respectively. Vascular proliferation and polymorphous infiltrate are among the overlapping features between PTCL-u and AITL.

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Hodgkin Lymphoma

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Hodgkin lymphoma (HL) is a clonal B-cell neoplasm as demonstrated by the detection of clonal immunoglobulin (Ig) V-gene rearrangements in isolated tumor cells using microdissection and single-cell polymerase chain reaction (PCR) techniques [1–5]. It is recognized by the current WHO classification as a malignant lymphoma with unique clinicopathologic features [6]. It affects more often the young adults with a median age of 38 years at diagnosis [7] and primarily involves lymph nodes commonly found in the supradiaphragmatic areas, and the neoplastic tissue comprises a minor population of large pleomorphic neoplastic cells (Hodgkin–Reed–Sternberg cells, or HRS cells, and their variants) that are admixed with an inflammatory background (Figures 18.1 and 18.2).

The incidence of HL is about 3 per 100,000 in Western Europe and the United States and is consistently lower than that of non-Hodgkin lymphoma (NHL). It accounts for about 10–15% of all lymphomas in Europe and the United States [6–10].

Based on its clinical behaviors, as well as morphologic, immunophenotypic, and genotypic profiles, HL is divided into two entities of nodular-lymphocyte-predominant HL (NLPHL) and classical HL (CHL) with the following subtypes according to the current WHO classification [6]:

- Nodular-lymphocyte-predominant Hodgkin lymphoma (NLPHL)
- Classical Hodgkin lymphoma
 - Nodular sclerosis classical Hodgkin lymphoma (NSHL)
 - Mixed cellularity classical Hodgkin lymphoma (MCHL)

- Lymphocyte-rich classical Hodgkin lymphoma (LRCHL)
- Lymphocyte-depleted classical Hodgkin lymphoma (LDHL).

NODULAR-LYMPHOCYTE-PREDOMINANT HODGKIN LYMPHOMA

Nodular-lymphocyte-predominant Hodgkin lymphoma (NLPHL) is a distinct but rare subtype of HL [11]. It represents 5–7% of all HL cases in the United States and Europe with an estimated 500 new cases each year in the United States [12]. NLPHL is a monoclonal B-cell lymphoma characterized by nodular pattern, presence of sparse large pleomorphic lymphocytic and histiocytic (L&H) cells (or popcorn cells, HRS cell variants) admixed with abundant B-lymphocytes that reside in an expanded meshwork of follicular dendritic cells. It most commonly presents as limited nodal disease involving peripheral lymph nodes above or below the diaphragm.

Etiology and Pathogenesis

The pathogenesis of NLPHL is poorly understood due, in part, to the rarity of the disease. Since NLPHL is virtually always Epstein–Barr-virus-negative, no EBV-related transforming event is known for the pathogenesis of NLPHL. A recent study shows aberrant somatic hypermutations (SHM) in the neoplastic cells of both CHL and NLPHL, which have been identified

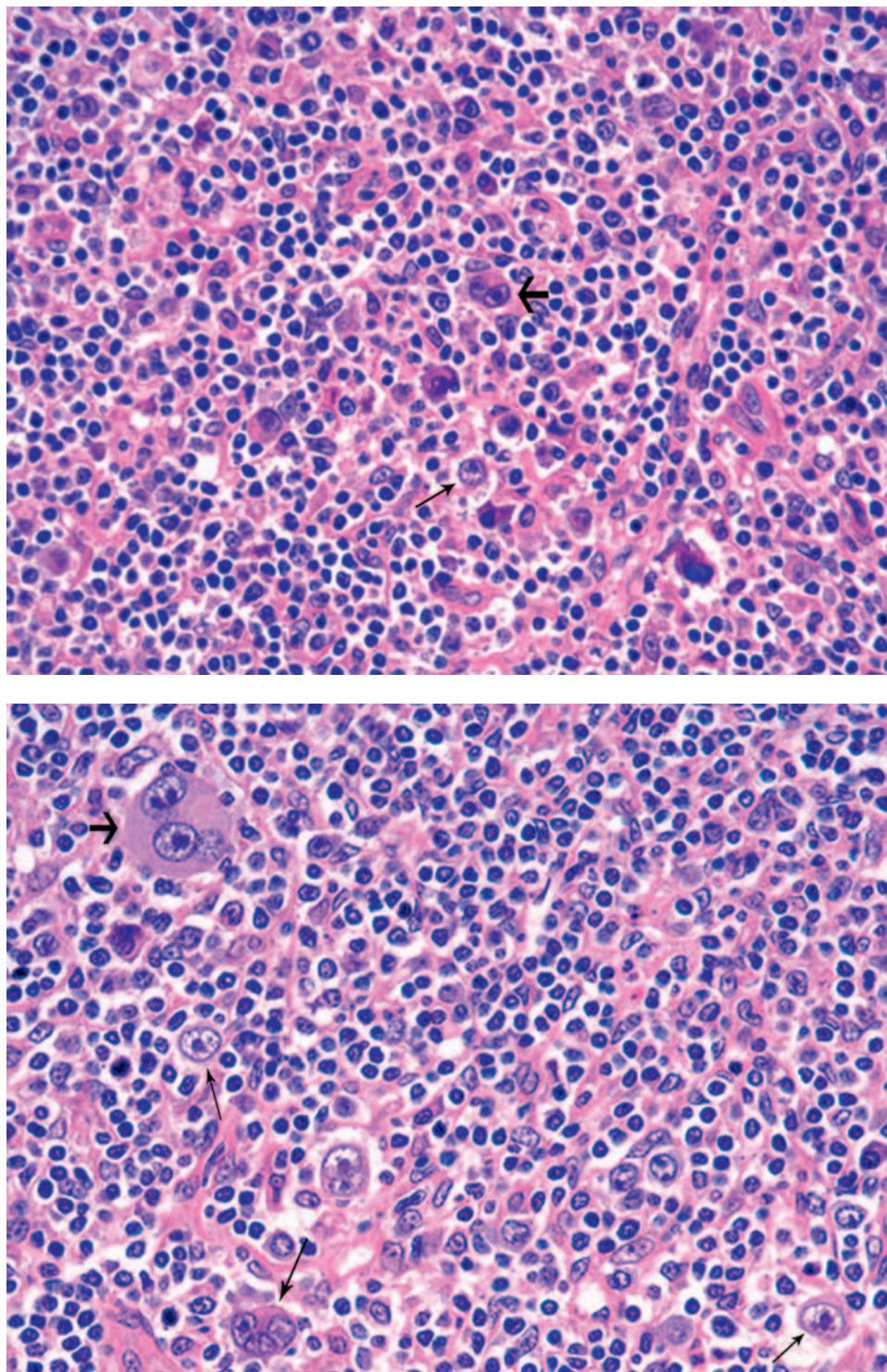


FIGURE 18.1 Reed–Sternberg cells (thick arrows) and Hodgkin cells (thin arrows) in a background of lymphocytes, plasma cells, and histiocytes.

in diffuse large B-cell lymphoma (DLBCL) as a mechanism for genome instability. This suggests common molecular pathogenetic events that are shared by these lymphomas [13]. In NLPHL, SHM of *SOCS1* (suppressor of cytokine signaling 1) loci is thought to be accompanied by high expression of *JAK2* and activation of the *JAK2*–*STAT6* pathway in the neoplastic cells [14].

Pathology

Morphology

Normal nodal architecture is partially to completely effaced by the neoplastic infiltrate, which, by definition, demonstrates at least partially nodular growth pattern (Figure 18.3). Residual follicles, follicular hyperplasia, and progressive

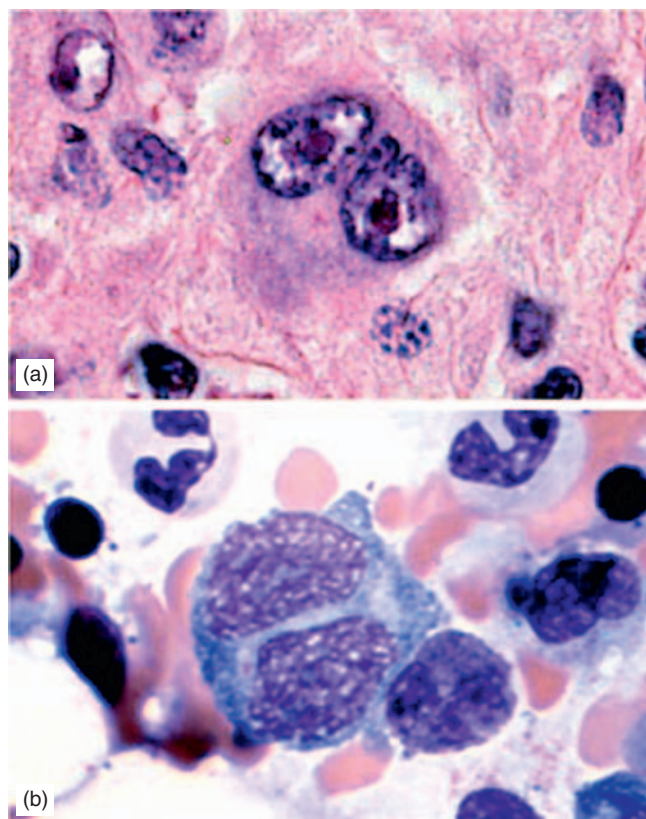


FIGURE 18.2 Classic Reed-Sternberg cells are large binucleated cells with prominent round nucleolar displaying an “owl-eye” appearance: (a) bone marrow biopsy section and (b) bone marrow smear.

transformation of germinal centers can be present simultaneously within the lymph node that is involved in NLPHL. The neoplastic nodules are usually large and poorly defined and are comprised of a minor population of scattered L&H cells, which are admixed with occasional single to clusters of histiocytes and abundant small lymphocytes that are typically B-cells (Figure 18.4). L&H cells are large and pleomorphic and often contain a single nucleus with folding and multilobation, resembling popcorn kettle and hence the name “popcorn” cells. L&H cells have vesicular chromatin, usually multiple small nucleoli with abundant basophilic cytoplasm but without distinct perinuclear halos. A follicular dendritic cell meshwork can be highlighted within the neoplastic nodules using special stains. When diffuse areas are present, they predominantly comprise T-cells as well as histiocytes with occasional L&H cells. Neutrophils, eosinophils, and plasma cells are not commonly seen. According to the current criteria, the detection of one nodule showing the typical features of NLPHL in an otherwise diffuse growth pattern is sufficient to exclude the diagnosis of T-cell/histiocyte-rich large B-cell lymphoma (T/HRBCL) [6].

Immunophenotype

The immunophenotypic profile of L&H cells is distinctly different from that of HRS cells [15, 16], and the diagnosis

of NLPHL should be confirmed by appropriate immunohistochemical studies [17]. L&H cells express CD45, pan-B-cell-associated antigens including CD20, CD22, and CD79a, as well as BCL-6, J-chain, and CD75. Epithelial membrane antigen (EMA) is positive in about 50% of the cases [18]. CD15 and CD30 are negative in nearly all cases, though weak expression of CD30 is rarely detected in L&H cells [18, 19]. Overexpression of BCL-2 is not seen, and EBV infection is generally not detectable either by immunohistochemical methods for latent membrane protein (LMP) or by *in situ* hybridization studies for Epstein-Barr early RNA (EBER) [20, 21].

Immunoglobulin light chain restriction is expressed in most cases [22], and heavy chains may also be positive [23]. L&H cells of a unique subset of NLPHL are positive for IgD exhibiting distinctive clinical features including striking male predominance, younger median age, and more frequent involvement of cervical lymph node [24]. This subset of NLPHL more often involves the interfollicular region in a background that is relatively rich in T-cells.

Recently, markers for B-cell transcription factors are used in distinguishing NLPHL from CHL, which include octamer-binding transcription factor 2 (Oct2), B-cell Oct-binding protein 1 (BOB.1), B-cell-specific activator, also known as PAX-5, and PU.1. L&H cells are positive for both Oct2 and BOB.1 and PAX-5 [23, 25]. The expression of PU.1 is found in most of the NLPHL cases [26, 27].

Staining for CD21 highlights expanded meshwork of follicular dendritic cells [28, 29]. The small lymphocytes seen in NLPHL are mostly polytypic B-cells admixed with occasional T-cells, some of which ring around L&H cells forming rosettes. These rosette T-cells can be highlighted by staining for CD3 or CD57 [30, 31]. Coexpression of BCL-6 and CD57 on rosette T-cells has been reported [32].

Cytogenetic and Molecular Studies

L&H cells in NLPHL represent transformed germinal center B-cells [4, 33]. There are only few reported cytogenetic and molecular mutations in NLPHL, including common rearrangements of the *BCL-6* gene [34] and that of SHM of SOCS1 loci. Because of the scattered nature of the neoplastic cells admixed with the non-neoplastic cells in HL, standard clinical molecular diagnostic assays (gene rearrangement clonality studies, etc.) are not of great use or, if performed, are usually negative because of the dilution effect of the background cell population. As noted at the start of this chapter, single-cell and microdissection PCR techniques have been used to prove the B-cell origin of neoplastic cells [4], but this is a research modality of largely academic interest. The same approach combined with DNA sequencing has been used to demonstrate the high rate of somatic hypermutation of the variable region genes, but again this is of scientific rather than practical diagnostic impact. Rearrangements of *BCL-6* are usually detected by immunohistochemical techniques rather than molecular tests, whereas the other chromosomal changes are best detected by cytogenetic techniques.

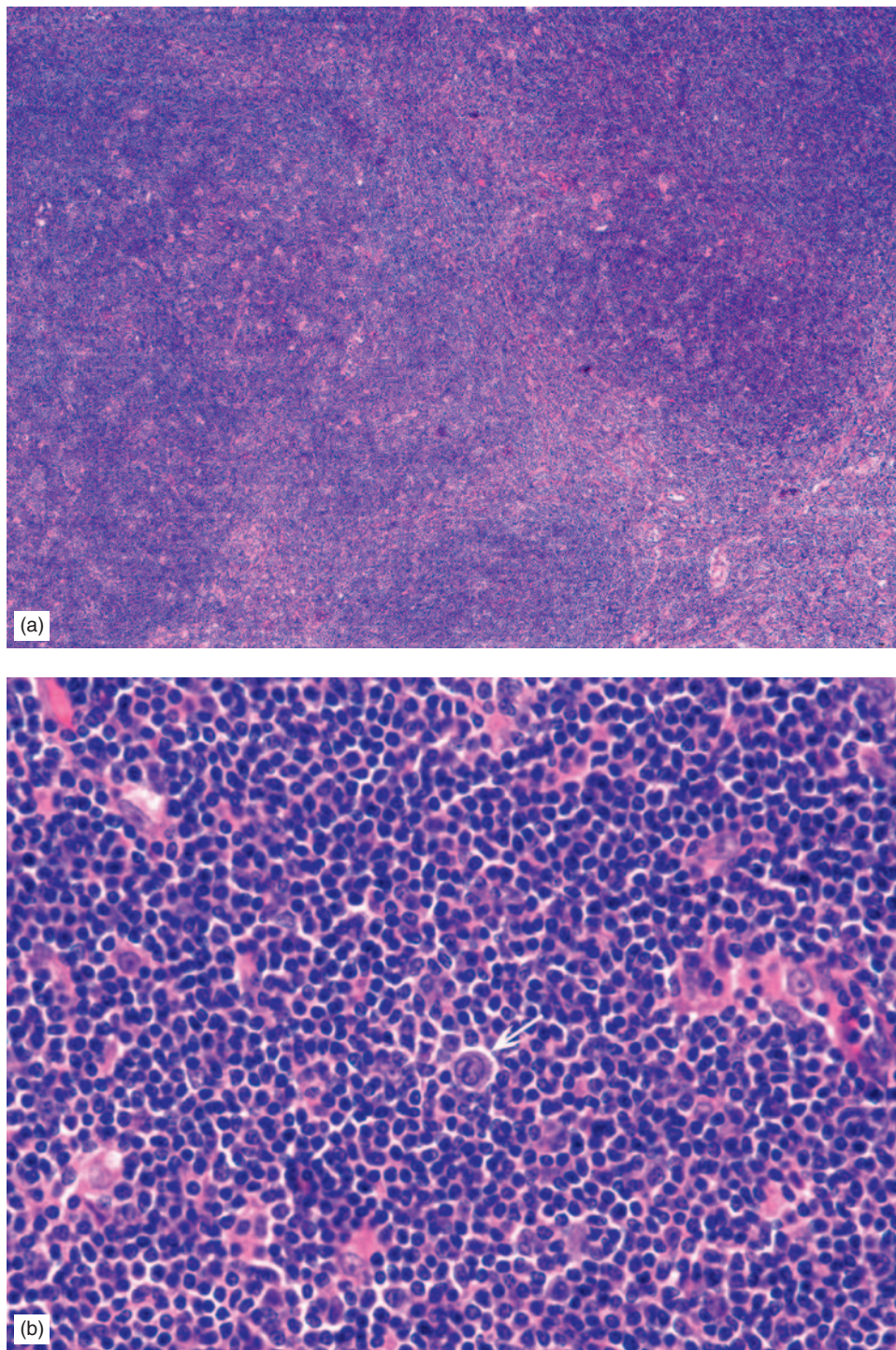


FIGURE 18.3 Nodular lymphocyte predominant Hodgkin lymphoma. (a) Low power view demonstrating a nodular pattern. (b) High power view showing an L&H cell (arrow) in a background of small lymphocytes.

Clinical Aspects

Patients with NLPHL are typically asymptomatic and present with localized peripheral lymphadenopathy frequently involving the cervical and axillary regions. Comparing with CHL, it is a more indolent disease with more frequent relapse, but good response to therapy, and overall more favorable prognosis. It is male predominant with a male:female ratio of 3–4:1 and has two peaks in age

distribution, one that of children and the other that of 30- to 40-year-old adults [12]. Most of the patients present with stages I and II diseases, and <20% of the patients with NLPHL present in stage III or IV disease, whereas approximately 50% of CHL patients present in more advanced stages [35–37]. The prognostically significant factors such as bulky disease and mediastinal involvement are rare in NLPHL.

Management of early stages of NLPHL includes the use of monoclonal antibody rituximab, involved field

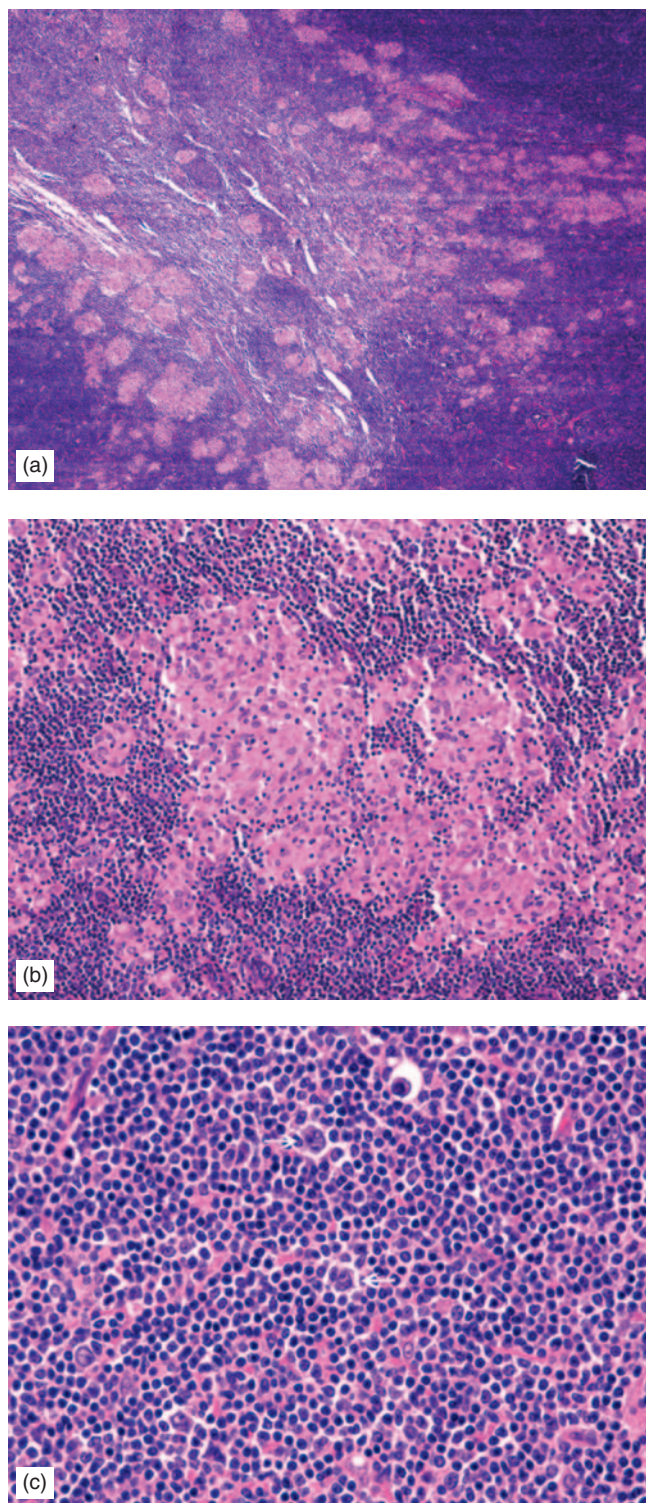


FIGURE 18.4 Nodular lymphocyte predominant Hodgkin lymphoma. (a and b) Histiocytic clusters are present mixed with small lymphocytes. (c) L&H cells (arrows) in a background of small lymphocytes.

or extended field radiotherapy, as well as “watch and wait” strategy [11, 38]. It is generally accepted that patients with early-stage NLPHL without risk factors can be treated with reduced intensity regimens resulting in less severe adverse

effects, whereas patients in early stage of disease with unfavorable risk factors are treated similar to CHL patients [38]. The treatment of NLPHL with standard HL protocols leads to complete remission in >95% of the patients [38]. Prognosis is worse in advanced-stage patients than in early-stage patients.

A very small proportion (2–7%) of NLPHL transforms to DLBCL [39–41], which has a more indolent course and more favorable prognosis than *de novo* DLBCL [40, 42].

Differential Diagnosis

The differential diagnosis of NLPHL includes T/HRBCL and lymphocyte-rich classical Hodgkin lymphoma.

T-cell/histiocyte-rich diffuse large B-cell lymphoma: Although NLPHL and T/HRBCL are two distinct lymphomas requiring different clinical management, they share overlapping morphologic and immunophenotypic features. Some studies suggest a biologic continuum between NLPHL and T/HRBCL [29], which implies that they may represent different spectrums of the same disease. Both lymphomas may have concurrent nodular and diffuse growth patterns, though by definition, at least partial nodular pattern has to be present for NLPHL. The immunophenotypic profile of L&H cells cannot be clearly distinguished from that of the neoplastic cells in T/HRBCL. However, immunophenotypic studies of the background composition are helpful [29], with common findings of abundant small B-cells and prominent follicular dendritic cell meshwork along with CD3+CD4+CD57+ T-cells in NLPHL, and lack of small B-cells but abundance of CD8+ T-cells and histiocytes in T/HRBCL.

Lymphocyte-rich classical Hodgkin lymphoma: NLPHL and LRCHL cannot be distinguished morphologically or clinically [37]. LRCHL frequently presents with a nodular growth pattern and a background of abundant small lymphocytes. The neoplastic cells in LRCHL sometimes display cytologic features of L&H cells seen in NLPHL. However, the immunophenotypic profile of the neoplastic cells in LRCHL differs from that of the L&H cells. The neoplastic cells in LRCHL show immunophenotypic features of HRS cells in other subtypes of CHL, including expression of CD30, CD15, Fascin, and EBV-LMP positivity in about 50% of the cases [17]. Unlike NLPHL, T-cell rosettes around the neoplastic cells generally do not express CD57 in LRCHL [18].

CLASSICAL HODGKIN LYMPHOMA

Classical Hodgkin lymphoma (CHL) represents about 95% of all HL cases in the United States and Europe. The estimated figure for the newly diagnosed cases of HL in 2007 in the United States was 8,190 [7]. CHL is a clonal B-cell lymphoma characterized by the proliferation of a minor population of mononuclear Hodgkin and multinucleated Reed–Sternberg (HRS) cells admixed with abundance of reactive

infiltrate including various inflammatory cells [6]. The diagnostic (classic) Reed–Sternberg cell is a large binucleated cell with prominent round nucleoli and perinucleolar halos displaying an “owl-eye” appearance (Figures 18.1, 18.2, and 18.5) [43]. Reed–Sternberg cell variants and mononuclear Hodgkin cells may display particular morphologic features in certain subclasses, such as “lacunar” cells in nodular sclerosis HL. It most commonly presents as lymphadenopathy involving the cervical, mediastinal, axillary, and para-aortic regions. Primary extranodal involvement is rare.

Etiology and Pathogenesis

Although the etiology is unclear, both genetic and environmental factors play a role in the pathogenesis of HL. Familial aggregation is found in approximately 4–5% of all cases of HL [44]. In addition, genetic predisposition of HL is supported by a significantly increased risk (99 times higher) of developing the disease in the identical twins of HL patients than in the dizygotic twins [45]. Furthermore, population-based data indicate ethnic variations with low incident rate of HL in Asian subgroups [46].

The genetic susceptibility of HL has been linked to human leukocyte antigens (HLA) for more than two decades [47], and many alleles and haplotypes have been implicated in either increased or reduced risks of the disease [48, 49]. More recent studies have further demonstrated the association of HLA class I with EBV+ HL, whereas HLA class III haplotypes are more associated with EBV–HL [50], and association of HLA-A*02 with reduced risk whereas HLA-A*01 with increased risk of developing EBV+ HL [51]. However, there is no consensus regarding the role of specific HLA alleles and haplotypes in HL due to the linkage disequilibrium of the HLA region [52].

HL is one of the EBV-associated lymphomas, and EBV infection appears to be the most significant environmental factor in the development of HL (Figure 18.5c). Several studies have implicated an increased risk of HL after infectious mononucleosis (IM) [53–55]. Recent studies suggest a causal association between IM-related EBV infection and the EBV+ subgroup of HL in young adults [56, 57]. Serologic analysis reveals elevated levels of IgG and IgA antibodies against EBV viral capsid antigen in patients with HL [58]. Immunohistochemical as well as fluorescence *in situ* hybridization (FISH) studies show EBV-positivity in approximately 30–40% of cases of HL [59]. Clonal EBV genome products can be detected in HRS cells in up to 50% of the CHL cases [60].

In addition to genetic predisposition and EBV infection, other factors including human immunodeficiency virus (HIV) infection, autoimmune disease, immunocompromised status, and existing lymphomas are associated with increased risks of HL [61–63].

Although the molecular pathogenesis of HL remains unclear, recent molecular studies have furthered our knowledge in understanding the pathogenetic mechanisms of HL. Aberrant activation of multiple signaling pathways in HRS cells is thought to play an important role in HRS cell survival and proliferation, which includes activation of Notch1, multiple receptor tyrosine kinases (RTKs), the PI3K,

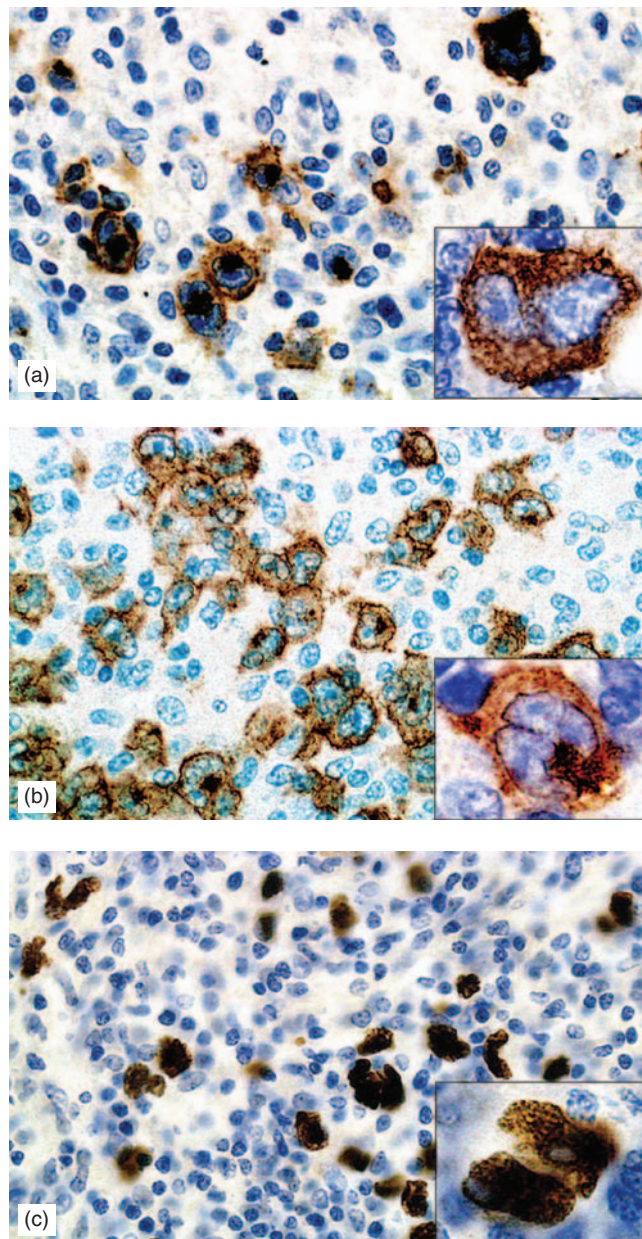


FIGURE 18.5 Reed–Sternberg and Hodgkin cells in classical Hodgkin lymphoma express: (a) CD15 and (b) CD30. They may also express EBV-encoded RNA, EBER (c and inset). Adapted from Ref. [43] by permission.

MED/ERK, NF- κ B, STAT, and AP-1 [64]. Among these pathways, constitutive activation of transcription factor NF- κ B is considered a hallmark of CHL [65–67]. Because EBV can be detected in up to 50% of the CHL cases, it is thought to contribute to the transforming mechanisms of HRS cells, and the pathogenesis of CHL is likely through pathways including viral-encoded gene latent membrane protein 1 (LMP1)-induced activation of NF- κ B [68] and LMP2A-driven B-cell survival in the absence of normal B-cell receptor signals [69]. In addition, genomic amplification of the c-REL, JAK2, and MDM2 loci, as well as expression of Caspase 3, is implicated in the antiapoptotic phenotype and survival of HRS cells [70–72]. Furthermore, the

microenvironment of HRS cells in CHL is uniquely favorable in protecting HRS cells from cell-mediated apoptosis, which is established and maintained by the unique cytokine profiles produced by HRS cells and specific regulatory T-cells [73].

Pathology

Morphology

There are four subtypes of CHL, which reveal distinct morphologic features [6], and are discussed in this section.

Nodular sclerosis classical Hodgkin lymphoma is the most frequent subtype of CHL accounting for 70–80% CHL cases in North America. It shows at least a focally nodular growth pattern with neoplastic nodules that are dissected by thick and fibroblast-poor collagen bands. Thickening of the capsule is often present. Scattered, large pleomorphic neoplastic cells including HRS cells as well as lacunar cells are admixed with abundant inflammatory cells (Figures 18.6 and 18.7). Lacunar cells, which are characteristic of NSHL, display paracellular halos as a result of retraction artifact of cytoplasmic membrane in formalin-fixed tissue. The number of HRS and lacunar cells is highly variable, as is the cellular composition of the reactive infiltrate in the background with small lymphocytes, eosinophils, neutrophils, plasma cells, histiocytes, and fibroblasts. Eosinophils and neutrophils can be abundant, and focal formation of eosinophilic or neutrophilic microabscesses can be seen.

Mixed cellularity classical Hodgkin lymphoma is the second most common subtype of CHL. It is more commonly seen in developing countries and has more frequent association with EBV infection and HIV/AIDS [61, 62]. The neoplastic infiltrate is mostly diffuse but can have a vaguely nodular pattern in focal areas. Even though interstitial fibrosis is seen, there should not be thickened fibrous capsule or broad collagen bands. The neoplastic infiltrate can present as interfollicular involvement accompanied by hyperplastic follicles in the background or regressed germinal centers. The neoplastic cells are typical HRS cells, which are admixed with highly variable reactive background (Figures 18.8 and 18.9). The reactive infiltrate in the background includes small lymphocytes, plasma cells, histiocytes, eosinophils, and neutrophils. Histiocytes can form granuloma-like aggregates.

Lymphocyte-rich classical Hodgkin lymphoma is a distinct subtype of CHL even though it has overlapping clinical and morphologic features with NLPHL. The neoplastic infiltrate displays more often a nodular but can be a diffuse growth pattern and is comprised of scattered HRS cells admixed with small lymphocytes. Eosinophils and neutrophils are absent. Occasionally, the neoplastic cells can resemble L&H cells seen in NLPHL.

Lymphocyte-depleted classical Hodgkin lymphoma is the least common subtype of CHL and is more frequently seen in developing countries and in persons with HIV/AIDS [17, 62]. Unlike the other subtypes of HL, the neoplastic infiltrate is diffuse and comprises relatively preponderant HRS cells. HRS cells can sometimes display

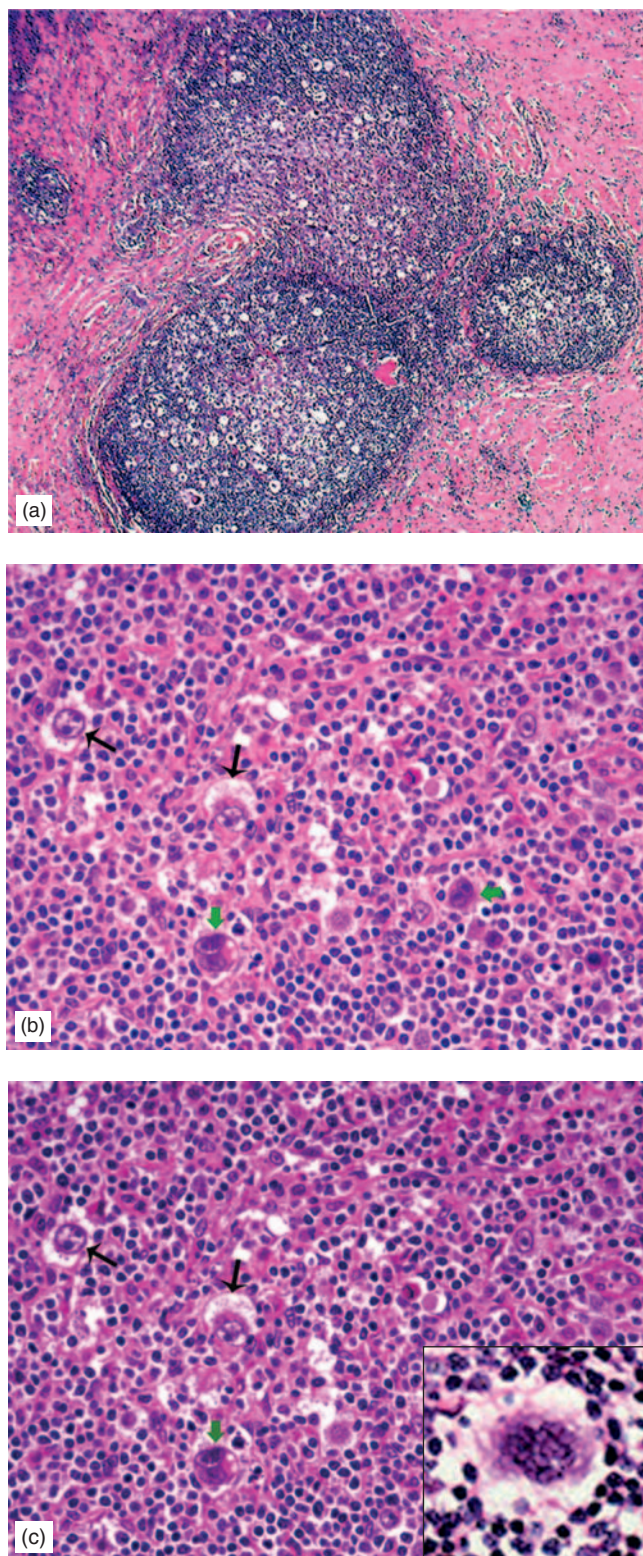


FIGURE 18.6 Nodular sclerosis Hodgkin lymphoma. (a) Low power view demonstrating a nodular pattern with numerous lacunar cells in the neoplastic nodules surrounded by thick collagen bands. (b and c) High power views showing lacunar cells (black arrows) and Reed–Sternberg cells (c, green arrows) mixed with lymphocytes and other inflammatory cells. The inset demonstrates a lacunar cell. Adapted from Ref. [43] by permission.

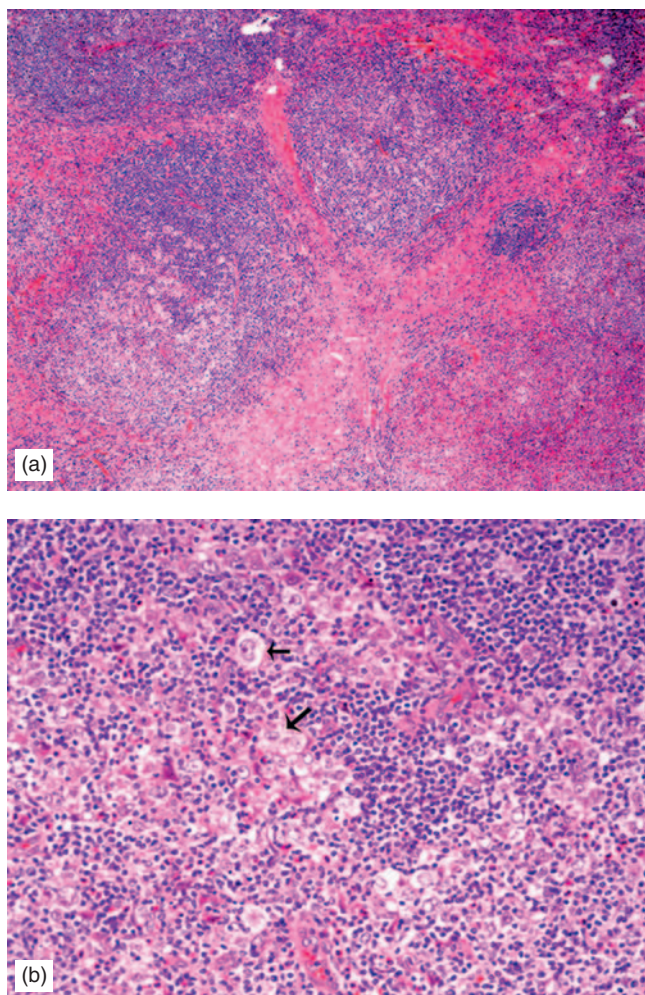


FIGURE 18.7 Nodular sclerosis Hodgkin lymphoma. (a) Low power view demonstrating a nodular pattern with the neoplastic nodules surrounded by thick collagen bands. (b) High power view showing a Reed-Sternberg cell (arrow) and several lacunar cells.

sarcomatoid appearance. The background contains relatively fewer numbers of small lymphocytes, and fibrosis can be prominent (Figures 18.10 and 18.11). If a nodular sclerosis is present, the lymphoma should be diagnosed as NSHL.

Immunophenotype

Hodgkin-Reed-Sternberg (HRS) cells are positive for CD30 and CD15 with membrane and Golgi staining, but are negative for CD45, J-chain, and CD75 (Figure 18.12) [22, 74, 75]. CD15 may be seen in only a minority of HRS cells. Fascin, an actin-bundling protein involved in the formation of dendritic processes, is positive in all CHL cases [76, 77]. Staining for B-cell-associated markers is variable, with positivity of PAX-5 in 90%, CD20 in 30–40%, and CD79a in 10% of CHL cases [22, 78, 79]. The expression of the B-cell-associated markers may be detected in a subpopulation of HRS cells and can vary in intensity generally revealing a weaker staining than that of the background B-cells [22, 80]. Unlike L&H cells of NLPHL where both

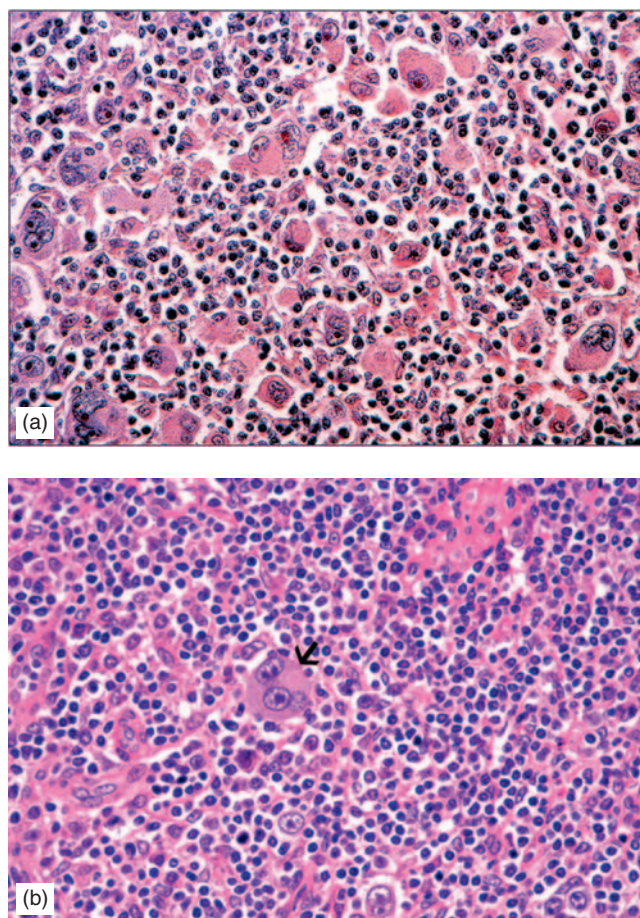


FIGURE 18.8 Mixed cellularity Hodgkin lymphoma. (a) Numerous Hodgkin and Reed-Sternberg cells are found in a background infiltrate predominantly consisting of lymphocytes. A classical Reed-Sternberg cell is demonstrated in (b, arrow).

Oct2 and BOB.1 are positive, HRS cells of CHL are negative for either Oct2 or BOB.1 [25]. BCL-6 nuclear staining of HRS cells is found in 40% of CHL cases [81]. MUM1 (multiple myeloma-1), a marker for late centrocytes and plasma cells, is detected in nearly all CHL cases, whereas it is absent or weakly positive in NLPHL [82–85].

HRS cells are positive for EBV infection in about 30–40% of the CHL cases by immunohistochemical as well as FISH studies [59]. EBV positivity is higher in MCHL and LRCHL than in NSHL [17, 60].

Cytogenetic and Molecular Studies

HRS cells are characterized as preapoptotic crippled germinal center B-cells that have lost their B-cell surface receptor expression [2]. HRS cells show cytogenetic abnormalities with numerical chromosomal changes, which commonly involve gains of chromosomes 2p, 12q, 17p, 9p, and 16p [86], indicating chromosomal instability of the HRS cells [87]. Two recurrent aberrations with gains of 2p13-p16 and 9p24 are identified in CHL, but not in NLPHL using comparative genomic hybridization analysis. Microarray analysis of

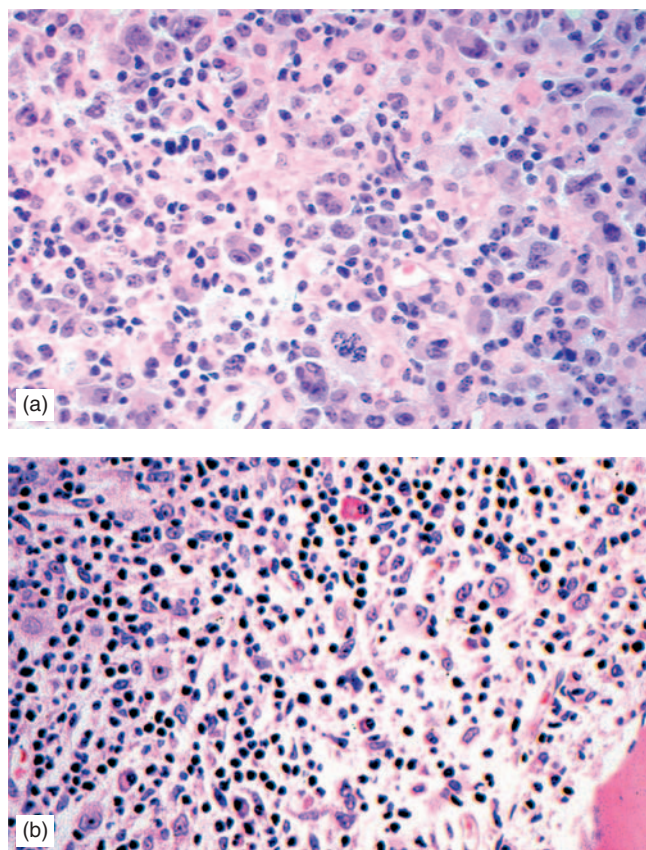


FIGURE 18.9 Mixed cellularity Hodgkin lymphoma; bone marrow biopsy section. (a) Numerous Hodgkin and Reed–Sternberg cells are present. (b) Scattered Hodgkin cells are mixed with numerous lymphocytes. Adapted from Ref. [43] by permission.

Hodgkin cell lines has identified expression of activating transcription factor 3 (ATF3), a member of the cyclic AMP response element binding protein (Figure 18.13) [88].

As in NLPHL, the molecular tests used for B- and T-cell non-Hodgkin lymphomas are not of much value in CHL. Sophisticated microdissection PCR techniques have again confirmed the neoplastic cells, and in particular, the Reed–Sternberg cells as being of B-cell origin; but these are not routinely used in the clinical setting. In contrast to NLPHL, somatic hypermutation of the immunoglobulin genes is not seen, whereas the *BCL-6* gene does show somatic mutations but no rearrangements or translocations [34, 89].

Clinical Aspects

The incidence of CHL is historically believed to have a bimodal curve with one peak in young adults (15–35 years of age) and the other at a late stage in life. According to the most recent statistics, the median age at diagnosis of HL is 38 years, and male:female ratio is slightly >1 [7]. Patients with a history of IM, HIV/AIDS, or autoimmune diseases have higher incidence of the disease [53, 62, 63]. Most of

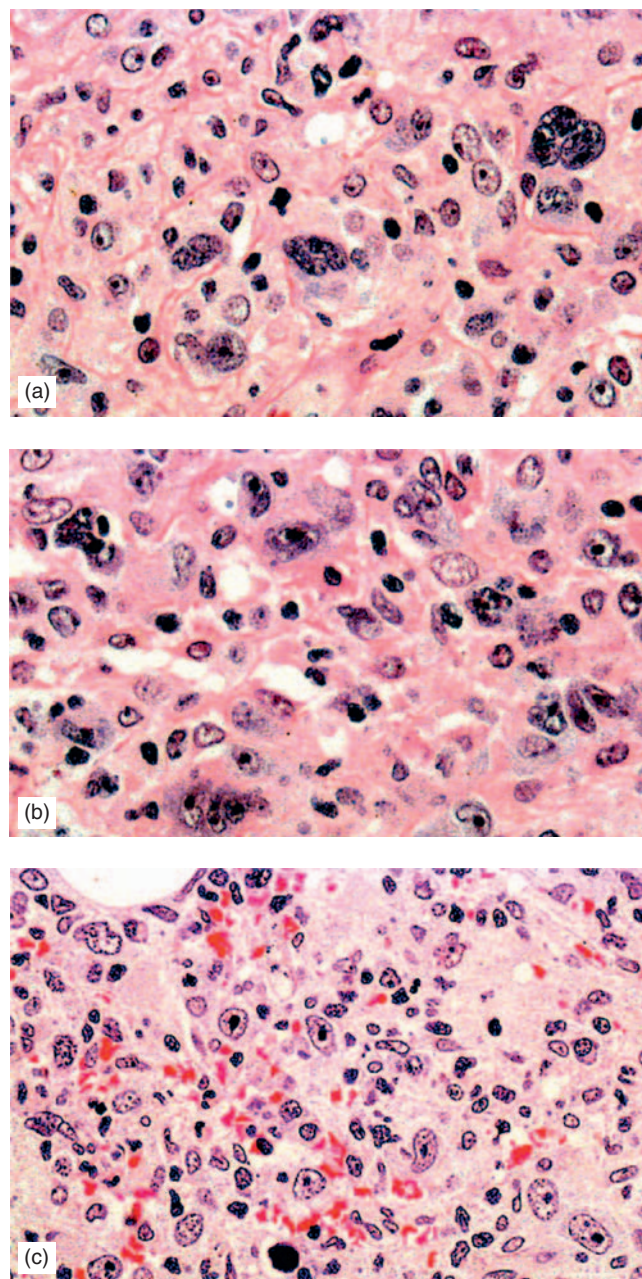


FIGURE 18.10 Lymphocyte-depleted classical Hodgkin lymphoma. Lymph node (a and b) and bone marrow biopsy (c) sections demonstrating numerous HRS cells mixed with scattered lymphocytes. Adapted from Ref. [43] by permission.

the CHL patients present with asymptomatic lymph node enlargement in the supradiaphragmatic regions. Systemic symptoms with fever, night sweats, and weight loss are reported in about 30% of the cases [10]. Approximately 50% of the patients have advanced-stage disease at diagnosis [35–37]. Splenic involvement is seen in about 20%, whereas bone marrow is involved in about 5% of CHL cases [6].

Staging of HL is based on the Ann Arbor system with the addition of the definition of bulky disease, which is currently favored by the maximum diameter of the largest

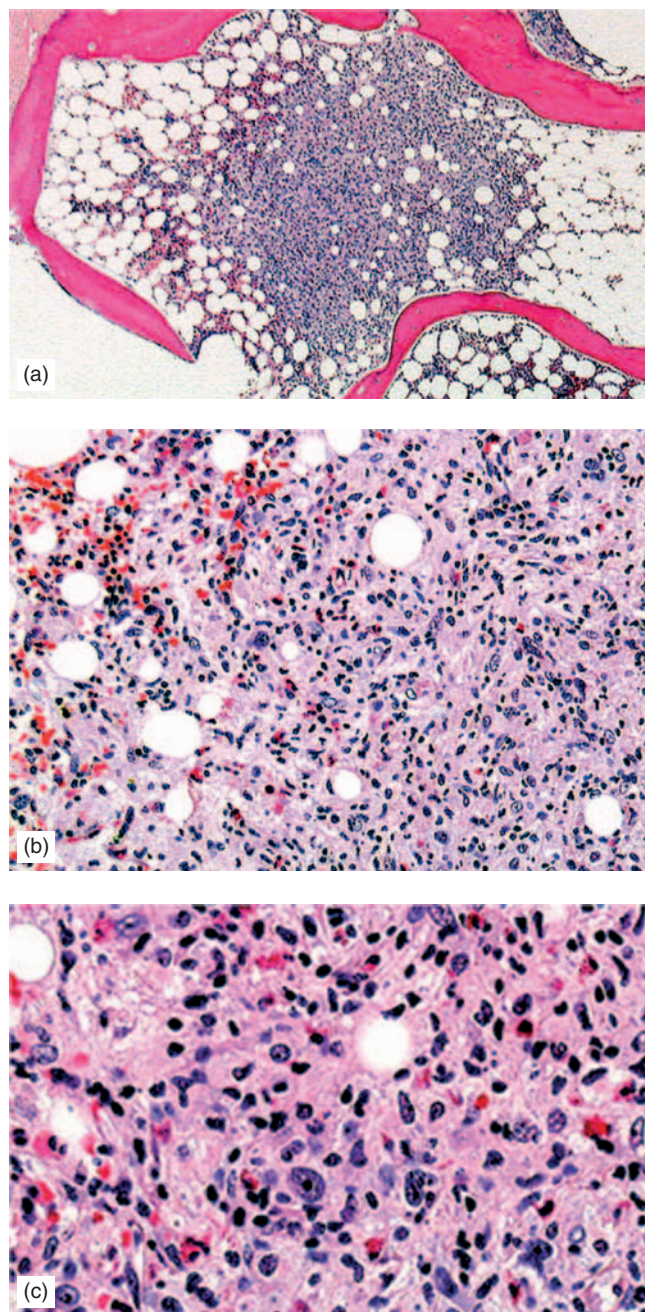


FIGURE 18.11 Bone marrow involvement in lymphocyte-depleted classical Hodgkin lymphoma demonstrating focal involvement with scattered HRS cells in a background of fibrosis and inflammatory cells: (a) low power, (b) intermediate power, and (c) high power views. Adapted from Ref. [43] by permission.

single tumor mass [90]. A limited-stage HL is usually defined by non-bulky stage IA or IIA disease, and a cure rate of >90% can be expected regardless of prognostic factor model. Seven independent prognostic factors have been identified, which are used to assess risks of primary treatment failure and possible intensified treatment in advanced-stage HL patients [91]. They include sex, age, stage, hemoglobin, WBC, lymphocyte count, and serum albumin.

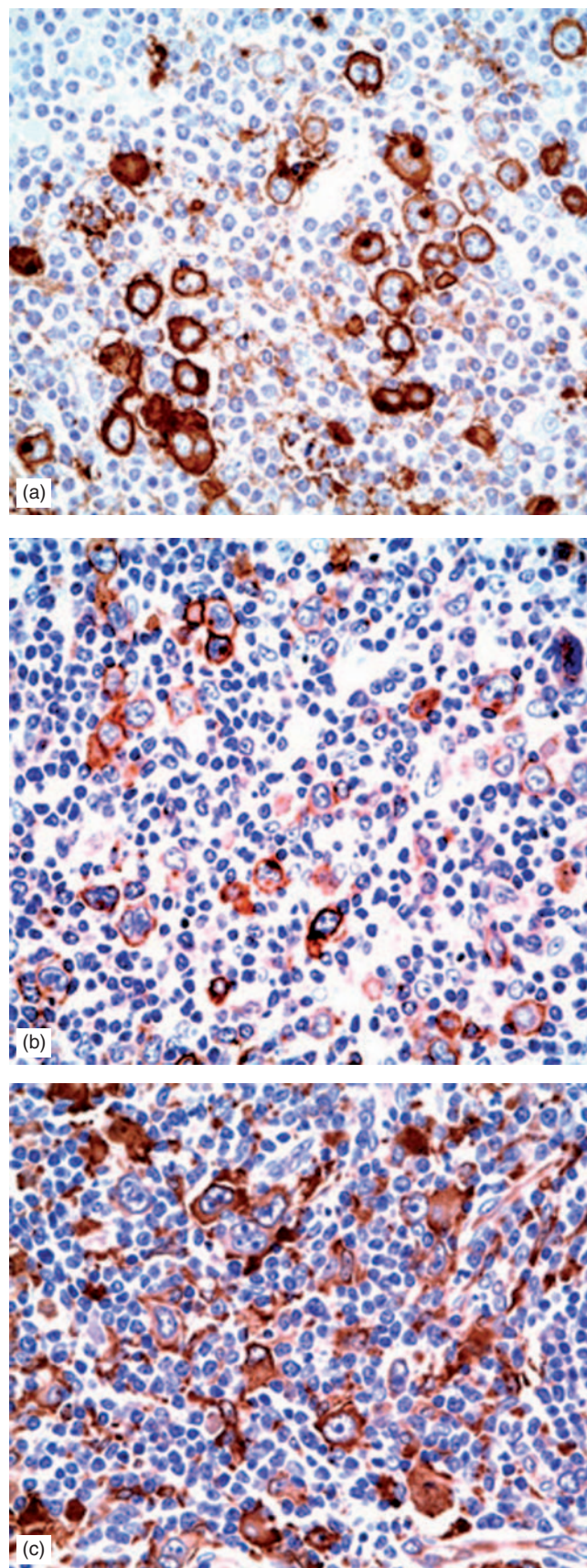


FIGURE 18.12 Immunohistochemical stains of HRS cells demonstrating positivity for (a) CD30, (b) CD15, and (c) Fascin.

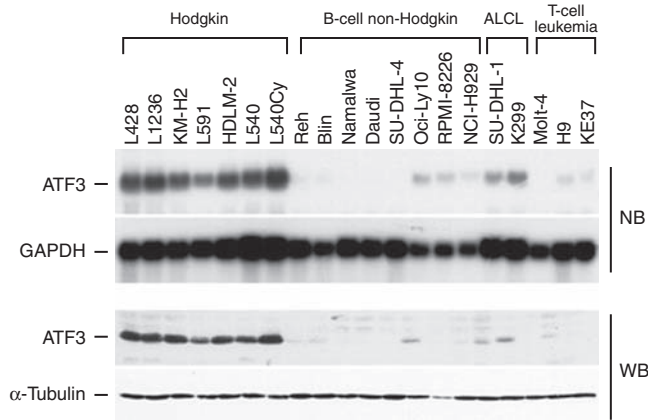


FIGURE 18.13 ATF3 mRNA and protein expression in HRS cells and Hodgkin and non-Hodgkin cell lines are determined by Northern blot and Western blot analyses. The Hodgkin cell lines and HRS tumor cells are characterized by high-constitutive ATF3 expression. Adapted from Ref. [88] by permission. This research was originally published in *Blood*.

Clinical characteristics vary among the distinct subtypes of CHL. Most patients of NSHL present with stage II disease, and mediastinal involvement is predominant [92]. In contrast to NSHL, MCHL often presents with stage III or IV disease and rare mediastinal involvement [6]. Unlike the other subtypes of CHL, LRCHL has clinicopathologic profiles resembling those of NLPHL with the exception that multiple relapse is less frequent [37]. LDHL is frequently associated with advanced-stage disease and more frequent involvement of abdominal organs, retroperitoneal lymph nodes, and bone marrow.

Treatment of HL represents one of the greatest success stories in modern medicine [93], and about 80–90% of patients in all stages achieve long-term survival [94]. The current understanding of the appropriate therapy is established on the basis of recent results of many large randomized clinical trials with focus on maximizing effectiveness while minimizing toxicity. For limited-stage HL, treatment involves brief, combined modality chemotherapy only augmented with involved field irradiation if an early CR is not achieved [93]. Patients with advanced-stage HL require extended course of chemotherapy without radiation therapy [95]. High-dose chemotherapy and irradiation and autologous hematopoietic stem cell transplantation can be an effective treatment for patients with relapsed or refractory HL [90, 96].

In addition to chemotherapy and radiotherapy, some studies suggest that immunotherapy including monoclonal antibodies play a positive role in the treatment of HL [97]. These suggestions are further supported by recent results of targeted immunotherapy using anti-CD30 chimeric receptors bound to EBV-CTLs that are persistent and can expand in HL patients for targeting the tumor and achieving complete patient response [98].

Concurrence of CHL and NHL is rare. Transformation of CHL is not well defined, even though cases have been reported where patients with CHL developed aggressive NHL including DLBCL and peripheral T-cell lymphoma [19, 99–101]. A clonal association

between the concurrent CHL and NHL has been demonstrated in some cases.

Differential Diagnosis

The differential diagnosis of CHL includes diffuse large B-cell lymphoma (DLBCL), anaplastic large cell lymphoma (ALCL), and senile EBV+ B-cell lymphoproliferative disorder.

Diffuse large B-cell lymphoma: Clinical and morphologic distinction can be difficult between CHL and large B-cell lymphoma, especially T/HRBCL, primary mediastinal (thymic) large B-cell lymphoma (PMBCL), and anaplastic variant of DLBCL. Appropriate immunophenotyping of the neoplastic cells is critical for making the distinction. The neoplastic cells of T/HRBCL are CD45+, EMA+, CD15–, and CD30–, whereas those of PMBCL are CD45+, CD15–, and can sometimes express CD30. Staining for Fascin can assist the immunophenotypic distinction between CHL and large B-cell lymphomas as it is mostly negative in large B-cell lymphomas but 100% positive in CHL [76, 77]. However, the differential diagnosis can sometimes be extremely challenging due to, perhaps, true biologic overlap between these entities [102, 103].

Anaplastic large cell lymphoma: Immunophenotypic analysis is helpful in differentiating CHL from ALCL. Although PAX-5 is positive in about 90% of CHL cases, it is consistently negative in ALCL [6]. Negative staining for EMA and ALK in CHL is also helpful. Furthermore, ALCL commonly expresses some T-cell-associated markers with clonal T-cell receptor gene rearrangements, which are extremely rare in CHL [104].

Senile EBV+ B-cell lymphoproliferative disorder: This recently described disorder represents a spectrum of diseases ranging from polymorphous B-cell lymphoproliferative disorders to DLBCL, and occasional cases may be indistinguishable from CHL on morphologic grounds [61, 105]. Immunophenotypic analysis can be helpful, and HRS-like cells in senile EBV+ B-cell lymphoproliferative disorder are positive for CD20, CD45, and EBV. They can be variably positive for CD30, but are negative for CD15 [106].

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Non-neoplastic and Borderline Lymphocytic Disorders

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In this chapter, the non-neoplastic lymphocytic disorders and lymphoproliferative disorders with borderline clinicopathological features (disorders of variable malignant potential) are discussed.

LYMPHOCYTOPENIA

Lymphocytopenia, or lymphopenia (absolute total blood lymphocyte count $<1,500/\mu\text{L}$), is one of the hallmarks of the primary and acquired immunodeficiency syndromes (AIDS). It also occurs in a wide variety of conditions such as aplastic anemia, tuberculosis, zinc deficiency, systemic lupus erythematosus, sarcoidosis, Hodgkin lymphoma (HL), toxic shock, and renal failure [1–4]. Administration of glucocorticoids and antilymphocyte globulin, cancer chemotherapy and radiotherapy, and thoracic duct drainage are frequently associated with lymphocytopenia.

Primary Immunodeficiency Syndromes

The primary immunodeficiencies are rare congenital disorders with defective function of the immune system leading to increased susceptibility to infection, autoimmunity, and development of malignant neoplasms [5, 6]. The primary immunodeficiencies include different subtypes representing T-cell (cellular) or B-cell (humoral) defects or combined deficiencies (Table 19.1). The T-cell defects are characterized by opportunistic viral and pneumocystis infections, whereas B-cell immunodeficiencies usually lead to bacterial infections [5]. In this section, severe combined

immunodeficiency (SCID), Wiskott–Aldrich syndrome (WAS), DiGeorge syndrome, and agammaglobulinemia are briefly discussed as examples of primary immunodeficiencies.

Severe combined immunodeficiency syndromes (SCID) consist of a heterogeneous group of disorders primarily arising from molecular T-cell defects leading to developmental and functional disturbances of T-cells and sometimes natural killer (NK) cells or B-cells (Table 19.2) [7–9]. There are two different modes of inheritance: X-linked and autosomal recessive. The X-linked form is associated with the mutation of the gamma chain of IL-2 receptor gene (*IL2RG*) and is characterized by reduced number of circulating T- and NK-cells and normal numbers of B-cells (Table 19.2). However, the B-cells do not mature to plasma cells, leading to a virtually non-existent serum immunoglobulin in these patients [7, 10]. Other variants of SCID are listed in Table 19.2. Of the many possible genes involved in SCID and related disorders, DNA sequencing to detect mutations is becoming available only for a few, such as *RAG1* and *RAG2*. Adenosine deaminase deficiency (ADD) is a subtype of SCID and accounts for about 50% of the autosomal recessive forms [11, 12]. ADD is characterized by a marked decline in the absolute number of both T- and B-cells in the peripheral blood and reduced number of hematogones and plasma cells in the bone marrow.

The typical symptoms of SCID are recurrent viral and bacterial infections, chronic diarrhea, and failure to thrive in newborns. These findings are associated with the lack of thymic shadow on chest X-ray studies. In some cases, clinical symptoms may be delayed by several months due to the protective effects of the maternally derived antibodies.

Wiskott–Aldrich syndrome (WAS) is a rare congenital disorder characterized by the triad of immunodeficiency, eczema, and abnormal

TABLE 19.1 Primary immunodeficiency syndromes associated with defective lymphocytes.*

Type	Inheritance	Molecular defect
T-cell defects		
SCID		
Reticular dysgenesis	AR	?
Allymphocytosis	AR	<i>RAG1; RAG2</i>
Deficit of T- and NK-cells	X-linked; AR	γ -chain- <i>IL-2R</i> ; <i>JAK3</i>
PNP deficiency	AR	<i>PNP</i>
Omenn syndrome	AR	5'-nucleotidase
T-cell activation defects (CID)		
HLA deficiency class-I	AR	<i>TAP2</i>
HLA deficiency class-II	AR	<i>CIITA-RFX5</i>
CD3 deficiency	AR	γ , ϵ CD3
Zap-70 deficiency	AR	<i>ZAP-70</i>
Calcium influx deficiency	AR	?
Defect in IL-2 synthesis	AR	?
Defects in DNA repair		
Ataxia telangiectasia	AR	<i>ATM</i>
Bloom syndrome	AR	<i>BLM</i>
Nijmegen syndrome	AR	<i>NBS1</i>
Xeroderma pigmentosum	AR	Complementation groups XPA to G
Others		
Wiskott–Aldrich syndrome	X-linked; AR	<i>WASP</i> ; ?
DiGeorge syndrome	Sporadic	del(22q11.2)/ <i>TBX1</i>
Hyper IgM syndrome	X-linked; AR	CD40 L; ?
B-cell defects		
Lymphoproliferative syndrome	X-linked; AD	?; <i>fas</i>
Bruton agammaglobulinemia	X-linked	<i>BTK</i>
Common variable immunodeficiency	Sporadic	?
IgA deficiency	AD; AR; sporadic	?
IgG subclass deficiency	AR	?
Hyper IgE syndrome	AR	?

*Adapted from Ref. [5].

AD: autosomal dominant, AR: autosomal recessive, BTK: Bruton tyrosine kinase, CID: combined immunodeficiency, PNP: purine nucleoside phosphorylase, SCID: severe combined immunodeficiency, WASP: Wiskott–Aldrich syndrome protein.

platelets (thrombocytopenia, small platelets, and platelet dysfunction) (see Chapter 24). Mutation of the *WASP* gene located on the chromosome Xp11.22-23 is the primary molecular defect. Numerous mutations have been reported, mostly found in exons 1 and 2 [13–15]. These mutations lead to the absence or aberrant expression of the WAS protein in lymphocytes and megakaryocytes. The WAS protein, a 53-kD cytoplasmic protein, appears to be involved in the regulation of actin in the cytoskeletal structures of T-cells and platelets. Lack of WAS protein expression may lead to decreased platelet size and defective T-cell and platelet function [5, 13].

WAS is an X-linked recessive disorder primarily involving males with an average age of about 21 months at diagnosis [16]. An autosomal dominant mode of inheritance has also been reported in some families [17], and complete sequencing of the *WAS* gene is available in a number of specialized laboratories to detect the mutations.

Clinical manifestations include bleeding secondary to thrombocytopenia and abnormal platelet function, recurrent infections, autoimmune manifestations, and eczema. There is an increased risk of hematologic malignancies,

TABLE 19.2 Gene defects and blood lymphocyte alterations in certain subtypes of severe combined immunodeficiency.*

Subtype	Gene	CD4	CD8	B	NK
X-linked, common γ -chain	<i>IL2RG</i>	↓	↓	N	
Janus kinase 3	<i>JAK3</i>	↓	↓	N	
IL-2 receptor- α (CD25)	<i>IL2RA</i>	↓	↓	N	N
IL-7 receptor- α (CD127)	<i>IL7RA</i>	↓	↓	N	N
CD3 complex	<i>CD3D</i>	↓	↓	N	
Recombinase activating genes	<i>RAG1, RAG2</i>	↓	↓	↓	N
Adenosine deaminase	<i>ADA</i>	↓	↓	↓	±
MHC class-II	<i>MHCIIID</i>	↓	N	N	N
Zeta-associated protein	<i>ZAP70</i>	N	↓	N	N
Protein tyrosine phosphatase (CD45)	<i>PTPRC</i>	↓	↓	N	

*Adapted from Ref. [9].

particularly non-HL [18]. The platelet counts are often $<50,000/\mu\text{L}$ and platelet size is usually half the normal size [19]. There is an inverse correlation between the severity of the clinical manifestations and the detectable levels of the WAS protein in the peripheral blood [20, 21]. The differential diagnosis includes immune-associated thrombocytopenia, other primary immunodeficiencies, myelodysplastic syndromes, and hematopoietic malignancies [22].

DiGeorge syndrome is a rare congenital immunodeficiency disorder characterized by the deletion of 22q11.2, developmental abnormalities of the third and fourth pharyngeal pouches, including thymic hypoplasia and hypoparathyroidism, and midline cardiac defects. The suggested criteria for definitive diagnosis of DiGeorge syndrome consist of reduced numbers of total CD3+ T-cells plus the presence of at least two of the following three findings [23]:

1. Deletion of chromosome 22q11.2 (Figures 19.1 and 19.2)
2. Hypocalcemia
3. Congenital cardiac defect.

Up to 90% of patients with DiGeorge syndrome show microdeletion of 22q11.2, and about 75% of the patients demonstrate congenital heart disease including tetralogy of Fallot, ventricular septal defect, or interrupted aortic arch [24–30]. As testing for the 22q11.2 deletion by fluorescence *in situ* hybridization (FISH) has become more widespread, the range of cardiac defects associated with the syndrome has expanded. Some centers have begun checking for the deletion in almost every patient with even an isolated congenital heart defect, and the diagnostic yield has been significant. Discovery of the deletion can then raise alertness to the possibility of other immunologic and endocrine complications (which are not present in all cases). Observation of the characteristic facial dysmorphism can also raise suspicion (the synonymous velocardiofacial syndrome). The deletion can also be detected in the context of a whole-genome scan for copy number variants (CNVs) using array comparative genomic hybridization (aCGH). The parents should also be

tested to distinguish *de novo* from inherited deletions, and to distinguish benign CNVs from pathologic deletions/insertions. Despite much work aimed at identifying the causative gene(s) in the deleted critical region, targeted molecular testing for mutations in the *TXB1* gene is not yet routine.

Agammaglobulinemia is one of the primary humoral immunodeficiencies and consists of two congenital types: X-linked and autosomal recessive [31–34].

The *X-linked* variant (Bruton agammaglobulinemia) affects boys with clinical manifestations between 6 and 18 months of age [32]. This disorder is caused by mutation in a tyrosine kinase gene called *BTK* (Bruton tyrosine kinase) mapped at Xq21 [35]. Complete sequencing of the *BTK* gene to detect these variants is available in several reference laboratories. The BTK product is a signal transduction molecule expressed in B-cells and other hematopoietic cells. The affected patients show profound hypogammaglobulinemia, reduced number of peripheral blood B-cells ($<1\%$ CD19+ or CD20+ cells), and recurrent bacterial and viral infections [32, 36]. An atypical form of X-linked agammaglobulinemia has been reported with less severe lymphopenia and hypogammaglobulinemia [37].

The clinical presentations of the *autosomal recessive* variant are similar to those of the X-linked variant except for the lack of *BTK* mutation and manifestation of the disease in both genders. The autosomal recessive type is caused by mutations in several genes that regulate B-cell development, such as *IGHM*, *CD179B*, *CD79A*, *BLNK*, and *LRRCB* [9, 38, 39].

Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome

The human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) has spread throughout the world. AIDS is associated with lymphocytopenia and defective-cell-mediated immunity leading to recurrent

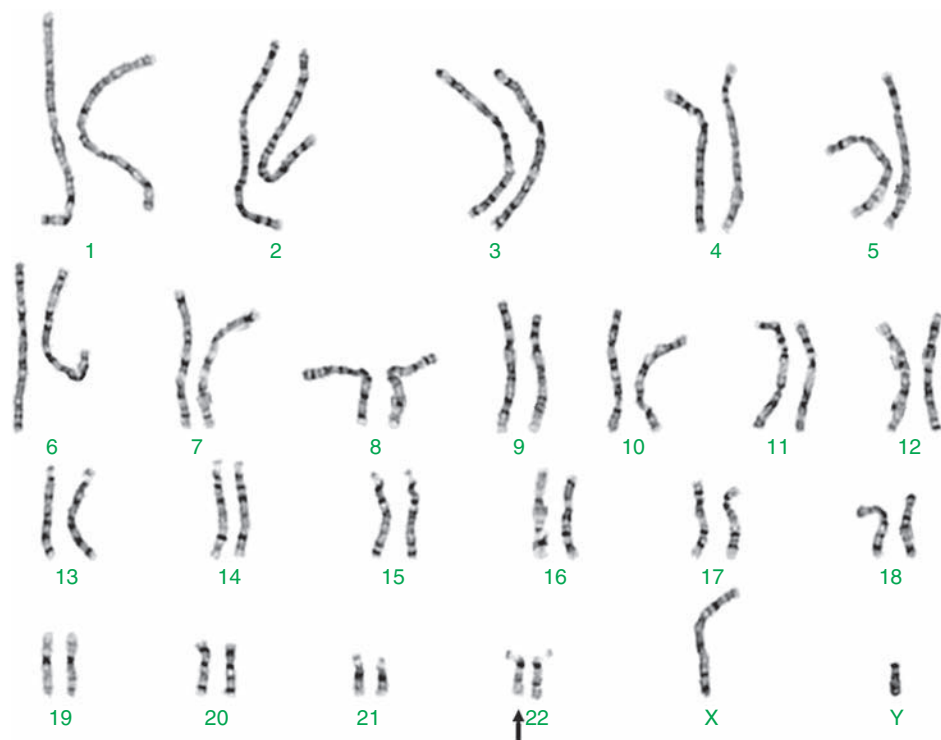


FIGURE 19.1 The characteristic karyotypic feature of DiGeorge syndrome is 22q11.2 (arrow).

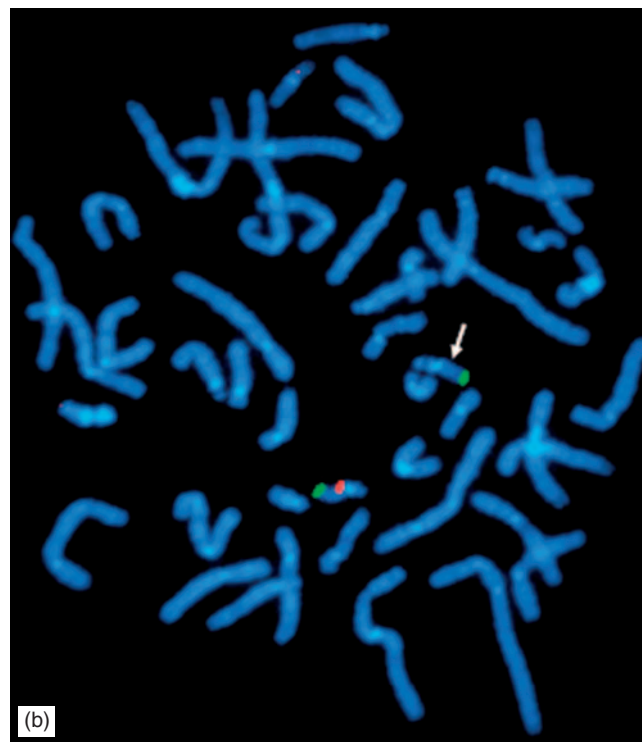
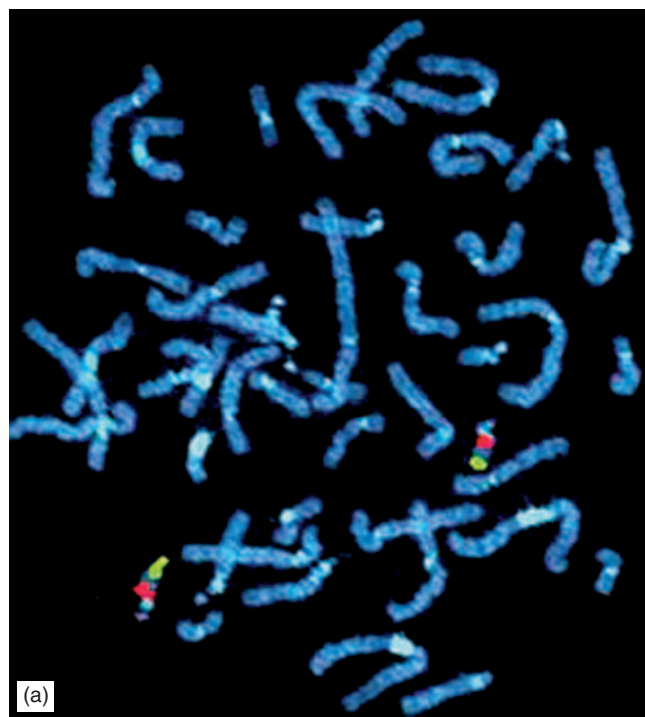


FIGURE 19.2 FISH analysis demonstrating a normal metaphase (a) and an abnormal metaphase with a subtle deletion of the 22q11.2 band (arrow) in a patient with DiGeorge syndrome (b).

infections, Kaposi sarcoma, lymphoma, and cervical cancer (Table 19.3) [40]. All HIV-positive patients with a CD4 cell count of $<200/\mu\text{L}$ are considered to have AIDS regardless of the presence or absence of clinical symptoms.

Etiology and Pathogenesis

AIDS is an HIV-induced illness with a broad spectrum of clinical manifestations. There are two strains of HIV: HIV-1 and HIV-2. HIV-1 was discovered first and

TABLE 19.3 Clinical conditions included in the 1993 AIDS surveillance.

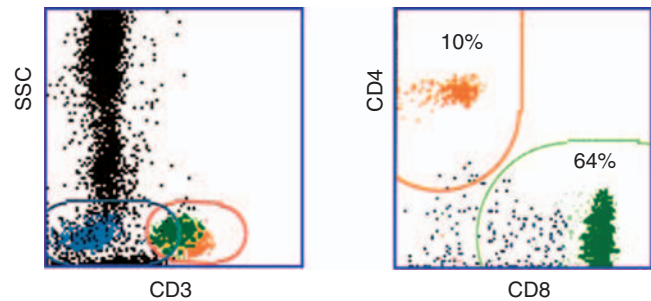
Candidiasis (respiratory system, esophageal)
Cervical cancer, invasive*
Coccidiomycosis, disseminated or extrapulmonary
Cryptococcosis, extrapulmonary
Cryptosporidiosis, intestinal
Cytomegalovirus disease (excluding liver, spleen, and lymph node involvements)
Herpes simplex infections, chronic
Histoplasmosis, disseminated or extrapulmonary
HIV encephalopathy
Isosporiasis
Kaposi sarcoma
Lymphoma (Burkitt, immunoblastic, primary brain)
Mycobacterium avium complex or kansasii, disseminated or extrapulmonary
<i>Mycobacterium tuberculosis</i> *
Mycobacterium, other species
<i>Pneumocystis carinii</i> pneumonia
Pneumonia, recurrent*
Progressive multifocal leukoencephalopathy
<i>Salmonella</i> septicemia, recurrent
Toxoplasmosis, brain
Wasting syndrome

Adapted from CDC, www.cdc.gov/mmwr/preview/mmwrhtml/00018871.htm

*Added in 1993.

is widespread in the United States, Europe, and other parts of the world, whereas HIV-2 is virtually homologous to the simian immunodeficiency virus (SIV) and is primarily detected in West Africa [41–43]. The general consensus is that HIV is the result of the cross-species transmission of SIV from African primates to humans [43].

HIV infection usually spreads through three major routes: sexual intercourse (70–80%), exposure to contaminated blood (5–10%), and prenatal transmission (5–10%) [44]. Viral penetration of mucosal epithelium is followed by infection of CD4+ T-cells, dendritic cells, and macrophages with subsequent spread to the lymph nodes and blood (viremia) [40, 45]. The viral envelope protein, GP-120, binds to CD4 expressed on T-cells, dendritic cells, and macrophages. The entry to the CD4+ cells is mediated by the chemokine receptor CCR5 [46]. Patients homozygous for a 32 base pair deletion in CCR5 are resistant to HIV infection [47], whereas heterozygous-infected individuals may have a

**FIGURE 19.3** Flow cytometry of peripheral blood demonstrating a population of CD3+ lymphocytes (absolute count = 980/ μ L) with reversed CD4:CD8 ratio (CD4 orange and CD8 green).

less aggressive clinical course. The heterozygote frequency in the general population is about 10% [48]. Although the molecular test for this deletion is straightforward, it has not found a place in routine HIV diagnosis or treatment.

The infected CD4+ T-cells are predominantly of the CD29+/CD45+ subset (memory cells). Several mechanisms have been suggested for the depletion and dysfunction of the infected cells, such as formation of syncytia (multinucleated giant cells from fusion of infected T-cells), cytotoxic effects of antiviral antibodies, release of cytotoxic cytokines, and inappropriate induction of apoptosis [49].

Pathology

The peripheral blood reveals lymphopenia (often <500/ μ L) with a reversed CD4:CD8 ratio (Figure 19.3) (CDC (1987). *MMWR* 36(suppl), 35). Scattered reactive lymphocytes may be present. Pancytopenia is observed in over 50% of the cases. Anemia is common and is usually normocytic normochromic, with decreased reticulocyte count and elevated serum iron and ferritin levels. Mild to moderate neutropenia is a common feature and approximately 30% of the patients demonstrate monocytopenia. Thrombocytopenia is frequent and in some cases it is autoimmune associated (ITP-like syndrome) [50].

HIV antibodies are raised against various viral components, such as envelope glycoproteins GP-120 and GP-41 and core protein p24. These antibodies are detected by a variety of techniques such as enzyme-linked immunosorbent assay (ELISA), Western blot, radioimmunoassay, and immunofluorescence [51]. Many patients show positive HIV serology (seroconversion) within 4–10 weeks after exposure to the virus, and >95% are seroconvert within 6 months [52, 53].

HIV RNA levels are detected in early stages of viremia by a sensitive reverse transcriptase-polymerase chain reaction (RT-PCR) assay [54, 55]. The detection of HIV RNA usually coincides with seroconversion [54, 55]. The quantitative RT-PCR assay is especially useful for monitoring viral load in patients on anti-retroviral therapy.

The bone marrow specimens from AIDS patients reveal a normocellular to hypercellular marrow, often with myeloid preponderance and left shift, and mild to severe dysplastic changes in one or more hematopoietic lines [56, 57]. An increased frequency of naked megakaryocyte nuclei has been observed in the bone marrow samples (biopsy sections

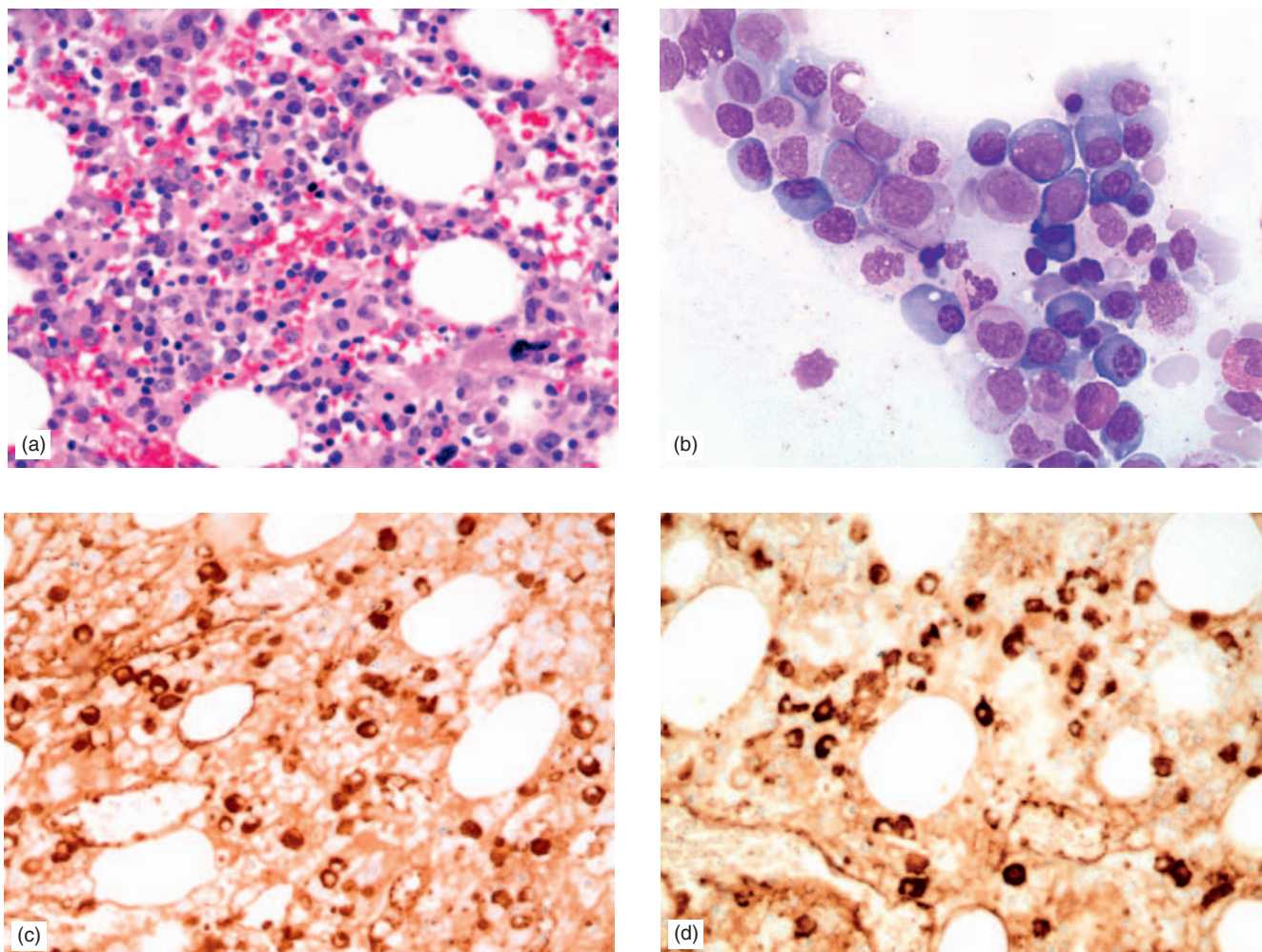


FIGURE 19.4 Bone marrow biopsy section (a) and smear (b) from a patient with AIDS showing a polyclonal plasmacytosis. Immunohistochemical stains for kappa light chain (c) and lambda light chain (d) demonstrate a polyclonal plasma cell population.

and marrow smears) of patients with AIDS [58]. A polyclonal lymphoplasmacytosis is common, sometimes with reactive lymphocytes and immature plasma cells (Figure 19.4). CD4⁺ cells are reduced, particularly those of the CD45RA-negative phenotype, whereas the proportion of CD8⁺ cells is increased, especially the HLA-DR⁺/CD45RA⁺ subtype [59]. Reticulin fibrosis and gelatinous transformation of bone marrow may be present.

The affected lymph nodes show various morphologic changes. Florid follicular hyperplasia is the most frequent pattern, particularly at the early stages of the disease. Numerous, large, irregular follicles with expanded germinal centers (GC), numerous mitotic figures, and tingible body macrophages are present [60]. Mantle zones are often ill-defined or effaced. The interfollicular areas show prominent vascularity and a mixed cellular component consisting of plasma cells, immunoblasts, histiocytes, lymphocytes, and monocytoid B-cells. The CD4:CD8 ratio is reversed. Sinus histiocytosis is a frequent feature and may be associated with hemophagocytosis [60].

In the later stages of the disease, there is evidence of lymphoid depletion by reduced number of the lymphoid follicles, decreased number of lymphocytes in the interfollicular areas, and the presence of amorphous eosinophilic deposits and/or fibrosis [60]. The affected lymph nodes may show signs of opportunistic infection – Kaposi sarcoma or lymphoma.

Clinical Aspects

The estimated number of people living with HIV in 2005 was 38.6 million with about 4.1 million newly infected ones and an estimated 2.8 million deaths due to AIDS (www.unaids.org, 2007). The major categories for HIV-1 infection include homosexual men, injection drug users, blood product recipients, and healthcare workers exposed to needle sticks [Trends in HIV/AIDS diagnoses: 33 states – 2001–2004 (225). *MMWR* 54, 1149–1153, <http://www.unaids.org>, 2004]. The major risk factors for the transmission of HIV infection include viral load, sexual intercourse, ulcerated

sexually transmitted diseases, nitrate inhalant use, and the host genetic background [44, 61, 62]. HIV infection is divided into several stages advancing from viral transmission to primary (acute) HIV infection, seroconversion, clinical latent period, early symptomatic stage, and finally to AIDS. The primary HIV infection is characterized by acute non-specific flu-like symptoms such as fever, headache, sore throat, myalgia/arthritis, diarrhea, nausea/vomiting, and lymphadenopathy. Clinical latent period refers to the 6-month asymptomatic period (except lymphadenopathy) after seroconversion. In this period, HIV is trapped by the follicular dendritic cells in the lymphoid tissues [63]. The early symptomatic stage (Class B), previously called “AIDS-related complex,” is associated with a number of clinical symptoms and opportunistic infections such as fever, chronic diarrhea, oral leukoplakia, peripheral neuropathy, thrombocytopenia, herpes zoster infection, vaginal candidiasis, and cervical dysplasia [44].

The revised 1993 definition of AIDS includes several clinical conditions (Table 19.3) plus a CD4⁺ lymphocyte count of <500/ μ L in HIV-infected patients. All HIV-positive patients with a CD4⁺ cell count of <200/ μ L are considered to have AIDS regardless of the presence or absence of clinical symptoms (www.unaids.org, 2007, www.cdc.gov/mmwr/preview/mmwrhtml/00018871.html). The most frequent clinical presentation associated with AIDS is *Pneumocystis carinii* pneumonia (42.6%) followed by esophageal candidiasis (15%), wasting (10.7%), and Kaposi sarcoma (10.7%) [44]. The spectrum and natural course of these complications have changed dramatically with the advent of anti-retroviral therapy.

Numerous drugs have been approved by the Food and Drug Administration (FDA) for the treatment of HIV infection including nucleoside analogs, protease inhibitors, and fusion inhibitors (www.niaid.nih.gov/factsheets/hivinf.html). Nucleoside analogs are nucleoside reverse transcriptase inhibitors, such as azidothymidine (AZT), zalcitabine (ddC), and stavudine (d4T). Examples for protease inhibitors include ritonavir (Norvir), indinavir (Crixivan), and saquinavir (Invirase). Fuzeon (Enfuvirtide) is the first approved fusion inhibitor drug.

Idiopathic CD4⁺ T-Lymphocytopenia

Idiopathic CD4⁺ T-lymphocytopenia is a condition characterized by acquired immunodeficiency, CD4⁺ lymphocyte depletion, and opportunistic infections *without* evidence of HIV infection [64–66]. These patients have an overall better prognosis than patients with AIDS, and a proportion of them may show a spontaneous regression [67]. The peripheral blood absolute CD4⁺ count is usually <300/ μ L.

LYMPHOCYTOSIS

Lymphocytosis is referred to the increased number of peripheral blood lymphocytes, >4,500/ μ L in individuals older than 12 years of age. The absolute lymphocyte count in children \leq 12 years may reach to as high as 8,000/ μ L in normal conditions [68]. Lymphocytosis is a common finding in most viral infections, certain bacterial infections,

TABLE 19.4 Major types of lymphocytosis.

Lymphoid malignancies
Virus-associated lymphocytosis
EBV
CMV
HIV-1
Hepatitis
Influenza
Measles
Mumps
Rubella
Varicella
Lymphocytosis associated with other infectious agents
<i>Babesia microti</i>
<i>Bartonella henselae</i> (cat-scratch fever)
<i>Bordetella pertussis</i> (Whooping cough)
<i>Brucella</i>
<i>Mycobacterium tuberculosis</i>
<i>Toxoplasma gondii</i>
<i>Treponema pallidum</i> (syphilis)
X-linked lymphoproliferative disease
Chronic polyclonal B-cell lymphocytosis
Polyclonal immunoblastic proliferation
Idiopathic lymphocytosis
Lymphocytosis associated with hypersensitivity reactions
Acute serum sickness
Drug-induced (e.g. ceftriaxone or carbamazepine)
Stress-induced lymphocytosis
Cardiac emergencies
Sickle cell anemia
Status epilepticus
Trauma
Other conditions associated with lymphocytosis
Autoimmune disorders
Cigarette smoking
Post-splenectomy
Thyrotoxicosis

X-linked lymphoproliferative (XLP) disease, post-splenectomy, thyrotoxicosis, certain lymphoid malignancies, and a number of other disorders (Table 19.4). Reactive lymphocytosis, particularly in viral infections, is associated with the presence of large, activated, or atypical lymphocytes. Atypical lymphocytosis is one of the characteristic features of infectious mononucleosis (IM) but has also been observed in other viral infections such as cytomegalovirus (CMV), varicella-zoster, rubella, and hepatitis.

Infectious Mononucleosis

Infectious mononucleosis (IM) is the clinical manifestation of Epstein-Barr virus (EBV) infection characterized by

fever, oropharyngitis, lymphadenopathy, and lymphocytosis with the presence of atypical lymphocytes in the peripheral blood [69, 70].

Etiology and Pathogenesis

EBV primarily spreads through saliva (kissing), infecting the epithelial cells of the oropharynx. The virus is replicated in the epithelial cells and released in the lymphoid-enriched surrounding environment, infecting B-cells through the EBV receptor CD21 (CR2, C3d receptor) [71]. The entry of EBV into the B-lymphocytes causes polyclonal B-cell proliferation. The EBV-transformed B-cells are able to induce a massive T-cell proliferation, primarily CD8⁺ and CD45RO⁺ cytotoxic T-cells [72, 73]. This rapid proliferation is associated with a short survival of CD8⁺ T-cells in the circulation [73]. Recent studies suggest that at least some degree of CD8⁺ cell proliferation is associated with the upregulation of Bim, a proapoptotic member of the Bcl-2 protein family and a major regulator of T-cell deletion [74].

Pathology

Lymphocytosis ($>4500/\mu\text{L}$) with the presence of more than 10% atypical lymphocytes is the characteristic morphologic feature of EBV infection. Atypical lymphocytes are large, pleomorphic cells with abundant shady gray-blue cytoplasm with or without vacuoles (Figures 19.5 and 19.6). They may show scalloping of the cytoplasmic membrane around red blood cells. The nucleus is round, oval, or irregular; the chromatin is clumped; and the nucleoli are often small or inconspicuous [75]. Anemia, granulocytopenia, and thrombocytopenia may occur, and leukocyte alkaline phosphatase (LAP) activity tends to be low [1].

Bone marrow examination reveals lymphocytosis with the presence of atypical lymphocytes. Small granulomas may be present, and there may be evidence of hemophagocytosis. The affected lymph nodes show expansion of paracortical regions with a population of polymorphous lymphoid cells ranging from small lymphocytes to large immunoblasts, mixed with tingible body macrophages and plasma cells [60]. Reed–Sternberg-like cells may be present. Other morphologic findings include small areas of necrosis, reactive follicles, dilated sinuses containing polymorphous lymphoid cells, and the presence of a polymorphous lymphoid infiltrate in the perinodal tissue.

The atypical lymphocytes represent activated lymphocytes that are predominantly of CD8⁺, CD45RO⁺, and HLA-DR⁺ phenotypes [76, 77]. The EBV-transformed immunoblasts are also present in the paracortical regions. The Reed–Sternberg-like cells may express CD30 but are negative for CD15 and EMA and positive for CD45.

The heterophil antibody test is positive, indicating the presence of cross-reacting antibodies to antigens from phylogenetically unrelated species, such as sheep (Paul-Bunnell test), equine (Monospot test), ox, and goat red blood cells [78, 79]. Specific antibodies against EBV antigens such as viral capsid antigen (VCA), EBV nuclear antigen (EBNA), and early antigen (EA) are detected [79]. EBV-encoded RNA (EBER) can be detected in the transformed cells by molecular techniques such as PCR assays and *in situ*

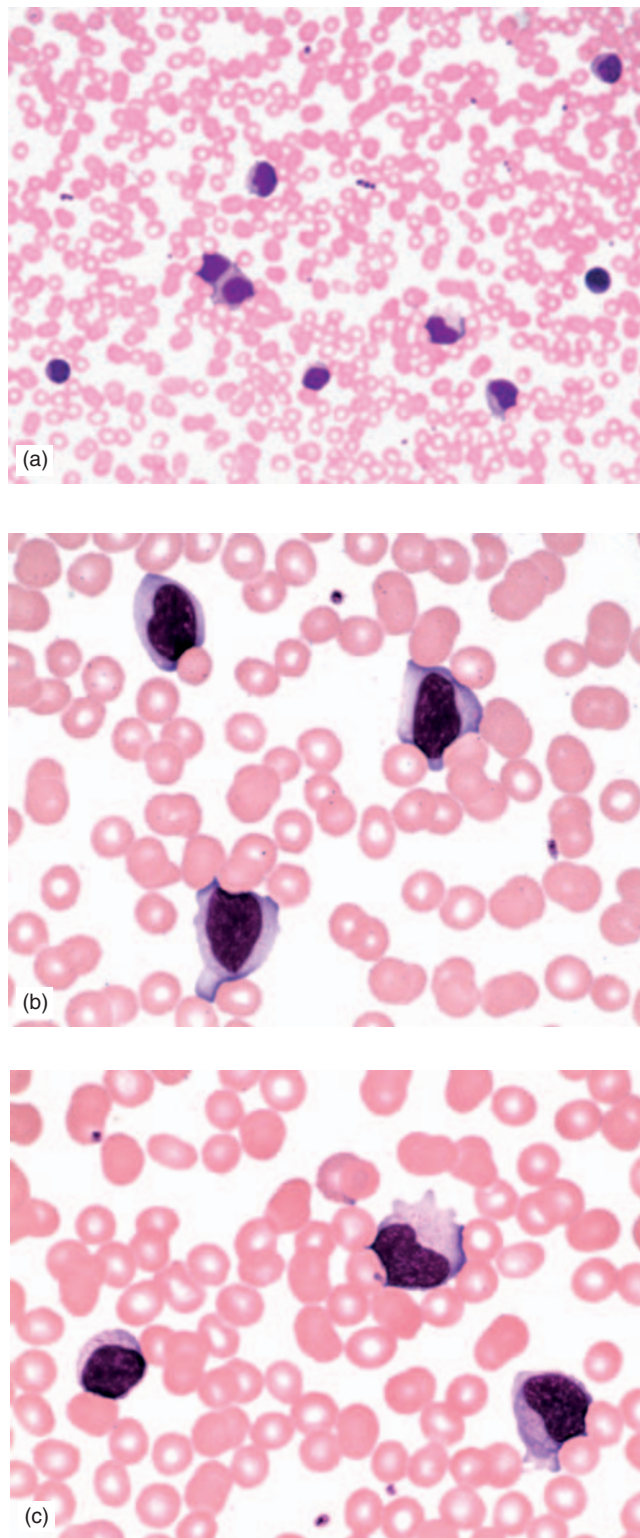


FIGURE 19.5 Blood smear from a patient with IM demonstrates large, pleomorphic atypical (activated) lymphocytes with abundant cytoplasm, round, oval, or irregular nucleus, and dense chromatin. The cytoplasm demonstrates some degree of basophilia and scalloping of the cytoplasmic membrane around erythrocytes. Some cells may show cytoplasmic granules: (a) low power, (b and c) high power views.

hybridization in the atypical lymphocytes or by immunohistochemical stains in the affected lymph node sections [80].

Clinical Aspects

Although EBV infects over 90% of the human population worldwide, the clinical manifestations of IM are uncommon in children and account for <3% of pharyngitis in adults [81]. The vast majority of primary EBV infections are not clinically detected [69].

Symptoms often begin with malaise, headache, and fever followed by lymphadenopathy and pharyngitis [82, 83]. Lymphadenopathy usually involves posterior cervical chains but could become systematic. It peaks in the first week and then gradually disappears within 2–3 weeks [69]. Other clinical findings include splenomegaly, neurologic symptoms such as facial palsies or meningoencephalitis, hepatitis, acute renal failure, and hemophagocytic lymphohistiocytosis [84–87]. IM is usually a self-limited disease and clinical symptoms disappear within 3–4 weeks. However, EBV infection in patients with X-linked lymphoproliferative syndrome may be fatal or lead to non-Hodgkin lymphoma. Also, rare cases of fatal T-cell lymphoproliferative disorders have been reported in association with EBV infection [88].

Supportive therapy is the recommended approach in treating patients with IM. Administration of antiviral drugs, such as Acyclovir, helps to protect people from EBV infection but has no effect on curing the infection [89].

Differential Diagnosis

Lymphocytosis with the presence of atypical lymphocytes is found in various conditions. Approximately 10% of the patients with the clinical symptoms of IM (atypical lymphocytes, fever, pharyngitis, and lymphadenopathy) are EBV-negative, and the condition is caused by other infectious agents, such as toxoplasmosis, CMV, human herpesvirus 6 (HHV-6), and hepatitis B [69, 90–92]. Some drugs such as phenytoin, carbamazepine, isoniazid, and minocycline may also cause atypical lymphocytosis [93, 94]. The diagnosis of EBV infection is confirmed by heterophil and/or specific EBV antibody tests or the identification of EBV by molecular studies, such as PCR assays [78–80]. However, one must exercise caution in interpretation of PCR results, given the high proportion of healthy EBV carriers in the population.

X-Linked Lymphoproliferative Syndrome

XLP syndrome is a rare inherited immunodeficiency characterized by lymphocytosis, dysgammaglobulinemia, fatal IM, or lymphoma usually developing in response to infection with EBV.

Etiology and Pathogenesis

Mutations in two X-linked genes, *SAP* and *XLAP*, have been reported in XLP patients [95–97]. *SAP* (*SH2D1A*), the most commonly affected gene, is located in the Xq25 region and encodes a signaling lymphocyte activation

molecule (SLAM)-associated protein. SLAM has a number of functions including regulation of T-cell cytotoxicity, T-cell/B-cell co-stimulation, and induction of interferon- γ in the Th₁ cells [98, 99]. SAP protein binds to the cytoplasmic tail of SLAM and also binds to other IG superfamily members, such as 2B4 expressed on NK-cells and cytotoxic T-cells [100, 101]. *SAP* mutations probably result in the defective T- and NK-cell responses and dysregulated cytokine release [98].

Mutation in *XLAP* (or *BIRC4*) gene has been recently reported in some patients with XLP who showed no evidence of *SAP* mutation. *XLAP* encodes the X-linked inhibitor of apoptosis. The *XLAP*-deficient patients with XLP have low numbers of NK-cells, suggesting that *XLAP* is required for the survival and/or differentiation of NK-cells [97].

Pathology

The most common pathologic finding is a massive, systemic lymphohistiocytosis associated with a clinical picture of fatal IM. The proliferating cells are EBV-infected B-cells and cytotoxic T-cells along with histiocytes. This lymphohistiocytic proliferation is accompanied by hemophagocytosis and dysregulated cytokine release resulting in extensive tissue damage, such as hepatic necrosis and profound bone marrow hypoplasia. The lymphohistiocytic infiltration has been described in other organs such as bone marrow, brain, heart, and kidney [98–102].

Approximately 35% of the affected children develop lymphoma, usually of B-cell type. Lymphoma is often extranodal and involves ileocaecal, central nervous system, liver, and kidney [103]. Burkitt lymphoma is the most frequent subtype (53%) followed by immunoblastic (12%) and follicular lymphomas (12%) [103]. HL is rare [98].

Abnormal production of serum immunoglobulin is a common finding and is often associated with a defective cellular immune function, presenting a picture of common variable immunodeficiency [104]. The degree of hypogammaglobulinemia ranges from moderately decreased levels of IgG to severe panhypogammaglobulinemia [105].

Clinical Aspects

XLP is a rare X-linked inherited disorder affecting boys with the age of onset ranging from 2 to 19 years [106]. The most common clinical presentation is a fatal IM following EBV infection, which has a very high mortality rate and a survival rate of <5% [98]. However, in approximately one-third of the affected patients, EBV infection is not fatal. These patients eventually develop dysgammaglobulinemia and/or lymphoma. The definitive diagnostic criteria include a male patient with lymphoma, fatal EBV infection, immunodeficiency, aplastic anemia or lymphohistiocytic disorder, and *SAP* mutation [102] (Table 19.5).

The treatment of choice is allogeneic hematopoietic stem cell transplantation [102]. Antiviral agents such as acyclovir or foscarnet, high dose immunoglobulin, immunosuppressive drugs, interferons α and γ , and HLH 94 have been tried with debatable outcomes [107, 108]. The HLH 94 (an antihistiocytic regimen) has been shown to induce long-term remissions.

TABLE 19.5 Diagnostic criteria for X-linked lymphoproliferative syndrome.*

<i>Definitive</i>
Male patient with lymphoma, immunodeficiency, aplastic anemia, lymphohistiocytic disorder, or fatal EBV infection and mutation in <i>SAP</i> gene.
<i>Probable</i>
Male patient with lymphoma, immunodeficiency, aplastic anemia, lymphohistiocytic disorder, or fatal EBV infection. Patient has maternal cousins, uncles, or nephews with a history of similar disorder.
<i>Possible</i>
Male patient with lymphoma, aplastic anemia, or lymphohistiocytic disorder, resulting in death, following EBV infection.

*Adapted from Refs. [98, 102].

Stress-Induced Lymphocytosis

A transient atypical absolute lymphocytosis with lymphocyte counts of up to 13,000/ μ L has been observed in adult patients with cardiac emergencies, trauma, status epilepticus, or sickle cell anemia crisis [68, 109–111]. The absolute lymphocytosis in these cases is usually the result of the increased numbers of B-, T-, and NK-cells [111].

Persistent Polyclonal B-Cell Lymphocytosis

Persistent (chronic) polyclonal B-cell lymphocytosis (PPBL) is a rare condition that has been reported in young to middle-aged women [112, 113]. An association with heavy smoking and HLA-DR 7 has been reported, suggesting that both environmental and genetic factors are involved [114, 115]. Reports of familial occurrences further support underlying genetic defects in this disorder [116, 117]. These individuals have absolute lymphocytosis ranging from 5,000 to 15,000/ μ L, with the presence of binucleated and/or atypical lymphocytes (Figure 19.7). There is a polyclonal increase in serum IgM levels with no lymphadenopathy or splenomegaly. The polyclonal B-cells express pan-B-cell markers such as CD19, CD20, and CD22 and show lack of or dim expression of CD5, CD10, and CD23 [114]. They may also express FMC7, CD11c, and CD25. In spite of its polyclonal nature and benign clinical behavior, PPBL, in some cases, has been associated with multiple *bcl-2*/Ig gene rearrangements and chromosomal abnormalities such as +i(3q), del (6q), and +8, respectively [115, 117, 118].

Polyclonal Immunoblastic Proliferation

Polyclonal immunoblastic proliferation is a rare, transient condition characterized by proliferation of B-immunoblasts and plasma cells in the lymph nodes, bone marrow, and peripheral blood [119, 120]. Hepatosplenomegaly and generalized lymphadenopathy are frequent clinical presentations. The etiology and pathogenesis are not clearly understood,

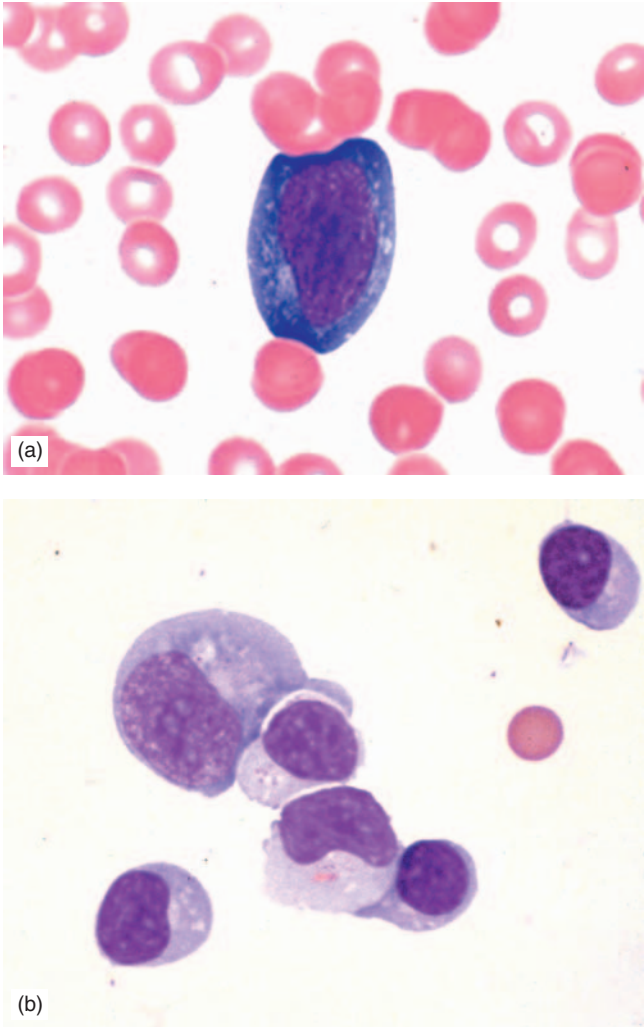


FIGURE 19.6 Blood smear (a) and pleural effusion (b) from a patient with IM showing atypical lymphocytes.

though an underlying abnormal immune response or viral infection has been suggested [119, 121]. Similar changes have also been associated with patients who receive methotrexate.

Bone Marrow Benign Lymphoid Aggregates

Benign lymphoid aggregates (lymphoid nodules, lymphoid follicles) are relatively frequent in bone marrow sections, ranging from <5% to >45% of the cases in various reports [122, 123]. They appear to be more frequent in older individuals and in women [124]. The presence of lymphoid aggregates in younger individuals usually indicates an underlying cause, such as autoimmune disorder, drug reaction, or viral infection. Benign lymphoid aggregates have also been reported in association with aplastic anemia, myeloproliferative disorders, myelodysplastic syndromes, mastocytosis, and HL and non-HL [125–128].

Lymphoid aggregates consist of small, well-defined clusters of mature lymphocytes that are sometimes mixed with scattered plasma cells, eosinophils, mast cells, or histiocytes (Figures 19.8 and 19.9). Lymphoid aggregates are

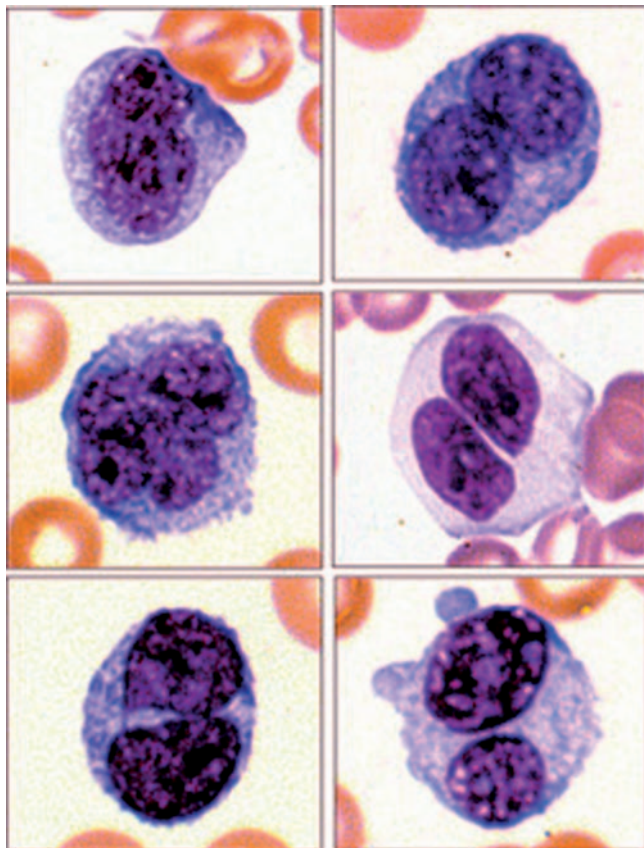


FIGURE 19.7 Binucleated lymphocytes and lymphocytes with lobated nuclei are frequently seen in patients with chronic PPBL. From Naeim F. (2001). *Atlas of Bone Marrow and Blood Pathology*. WB Saunders, Philadelphia, by permission.

usually interstitial, surrounded by fat or hematopoietic cells. They are usually distant from bone trabeculae. Approximately 5% of lymphoid aggregates may show germinal centers. Their presence may indicate reaction to a marked or prolonged immunologic stimulation. The term *lymphoid nodular hyperplasia* is used when four or more lymphoid aggregates are seen in a low power microscopic field, or if an aggregate exceeds 0.6 mm in its greatest dimension [124]. *Reactive polymorphous lymphohistiocytic lesion* refers to aggregates consisting of a mixture of lymphocytes, histiocytes, and other inflammatory cells [122]. These lesions may be large, ill-defined, and paratrabeular.

Differentiation of benign lymphoid aggregates from lymphomatous involvement in the bone marrow is sometimes problematic [129] (Table 19.6). Benign lymphoid aggregates are often well defined, lack an infiltrative pattern, are not paratrabeular, and are primarily composed of small, mature lymphocytes with round nuclei and condensed chromatin. They consist of a mixture of B- and T-cells with no evidence of monoclonality based on immunophenotypic, molecular, and/or cytogenetic studies. The B-cell component of the lymphoid aggregates is negative for bcl-2 and usually lacks the expression of CD5, CD10, and CD23 (Table 19.7) [130–132].

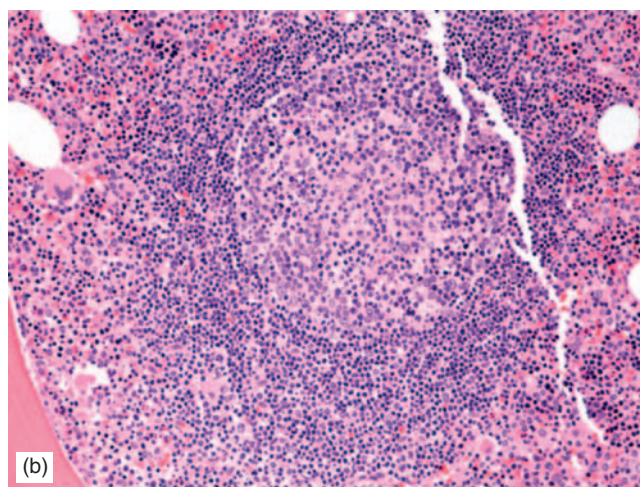
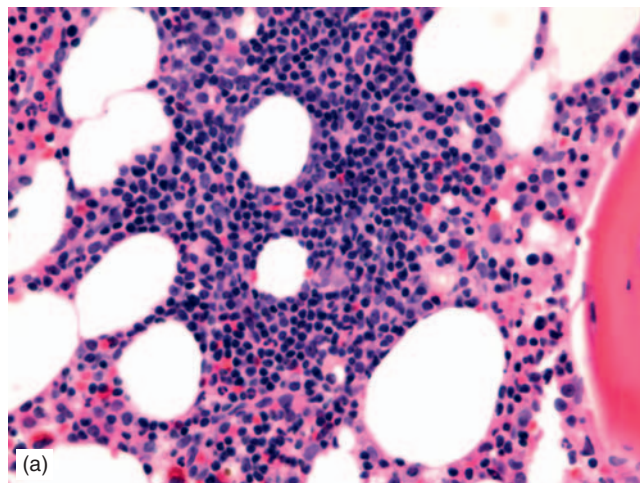


FIGURE 19.8 Benign lymphoid aggregates in bone marrow. Biopsy sections demonstrating lymphoid aggregates (a and b), one with germinal center (b).

LYMPHOPROLIFERATIVE DISORDERS OF VARIABLE MALIGNANT POTENTIAL

Post-transplant Lymphoproliferative Disorders

Post-transplant lymphoproliferative disorders (PTLD) are benign or malignant lymphoid disorders which develop after solid organ or bone marrow allogeneic transplantation [133, 134]. They represent a complex group with a wide spectrum of clinicopathological features ranging from lymphoid hyperplasia to full-blown lymphoma. The World Health Organization (WHO) classification defines four major categories: (1) early lesions, including reactive plasmacytic hyperplasia and IM-like PTLD, (2) polymorphic PTLD, (3) monomorphic PTLD (lymphoma), and (4) Hodgkin lymphoma and Hodgkin lymphoma-like PTLD [133] (Table 19.8).

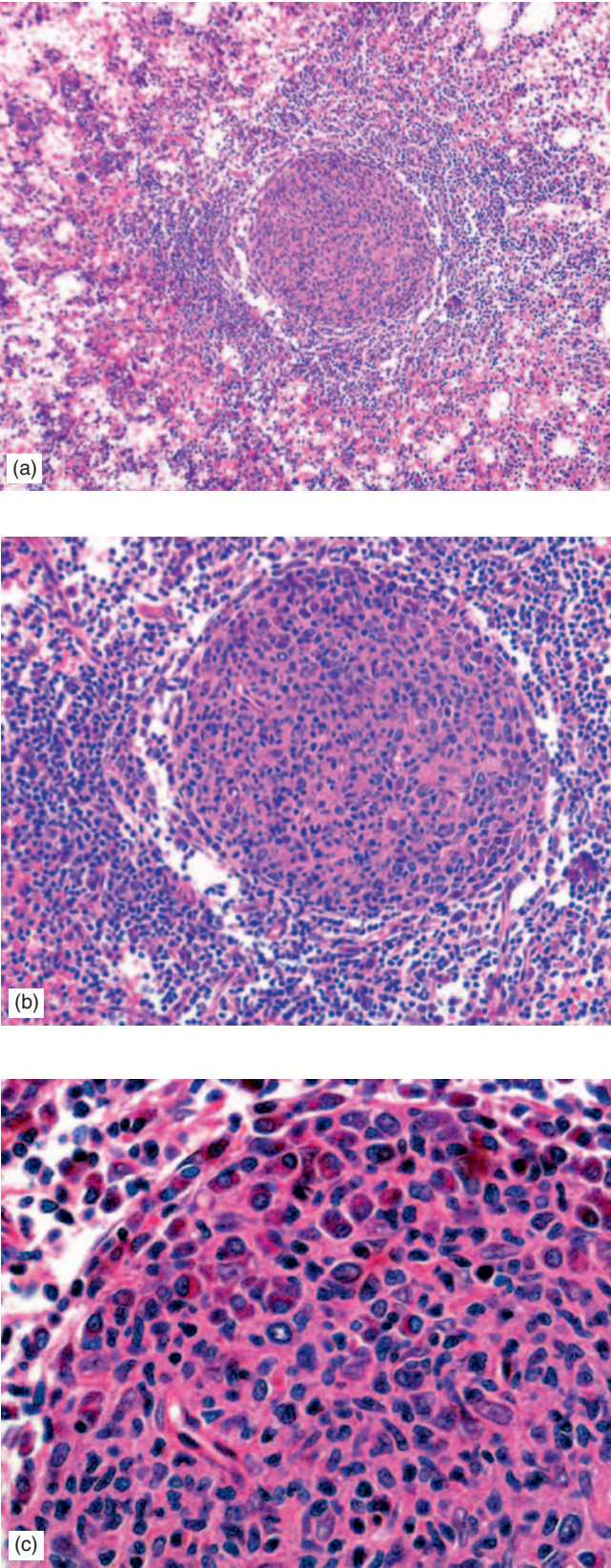


FIGURE 19.9 Bone marrow biopsy section from a patient with AIDS demonstrating an atypical lymphoid aggregate with germinal center: (a) low power, (b) intermediate power, and (c) high power views.

TABLE 19.6 Features of benign and malignant lymphoid aggregates in bone marrow sections.

Benign lymphoid aggregates	Malignant lymphoid aggregates
Often well defined and circumscribed	Usually irregular and infiltrating into the adjacent marrow
Usually interstitial	Frequently paratrabecular
Infrequent cellular atypia	Common cellular atypia
Germinal centers may be present	Germinal centers are not present
Polymorphous lesions lack Reed–Sternberg cells and Reed–Sternberg variants	Presence of Reed–Sternberg cells and variants in Hodgkin lymphoma
Lack of significant fibrosis	May be associated with significant fibrosis
B-cells in aggregates are usually negative for bcl-2, CD5, CD10, and CD23	Malignant B-cells often express bcl-2 and may also express CD5, CD10, or CD23
No evidence of monoclonality by immunophenotypic, molecular, and/or cytogenetic studies	Non-Hodgkin lymphomas often show evidence of monoclonality

TABLE 19.7 Conditions associated with benign lymphoid aggregates in the bone marrow.

Autoimmune disorders
Rheumatoid arthritis
Systemic lupus erythematosus
Autoimmune hemolytic anemia
Idiopathic thrombocytopenia
Hashimoto thyroiditis
Myelodysplastic syndromes
Myeloproliferative disorders
Mastocytosis
Aplastic anemia
Lymphoid malignancies
Viral infections
Drugs
Unknown

Etiology and Pathogenesis

The primary predisposition conditions associated with PTLT are immunosuppression and EBV infection [135–137]. EBV infection is documented in 50–80% of patients with PTLT cases. The EBV-negative cases are mostly renal allograft recipients which tend to occur later than the EBV-positive cases (>5 years after transplantation) [138, 139]. The vast majority of the PTLT cases (>90%) in the solid

TABLE 19.8 WHO categories of post-transplant lymphoproliferative disorders.*

Type	Major characteristic features
<i>Early lesions</i>	
Reactive plasmacytic hyperplasia	Preserved nodal architecture, increased plasma cells, and rare immunoblasts
IM-like lesions	Preserved nodal architecture, paracortical expansion with numerous immunoblasts.
Polymorphic PTLD	Destructive infiltrates consisting of a mixture of small and large lymphoid cells and plasma cells. Scattered atypical cells, areas of necrosis, and frequent mitotic figures may be present. Rearrangement of Ig or presence of EBV genome. Lack of mutations of <i>MYC</i> , <i>RAS</i> , and <i>TP53</i> genes.
Monomorphic PTLD	Destructive infiltrates of monomorphic atypical lymphoid cells consistent with lymphoma. Most frequent B-cell types are diffuse large B-cell, Burkitt lymphoma, and plasma cell myeloma. Rearrangement of Ig or presence of EBV genome. Mutations of <i>MYC</i> , <i>RAS</i> , and <i>TP53</i> genes may be present. Most frequent T-cell type is peripheral T-cell lymphoma, not otherwise specified. TCR gene rearrangement and up to 25% clonal EBV genome.
Hodgkin lymphoma (HL) and HL-like PTLD	Presence of classical Reed–Sternberg cells expressing CD15 and CD30. Presence of Reed–Sternberg-like cells and morphology consistent with lymphoma.

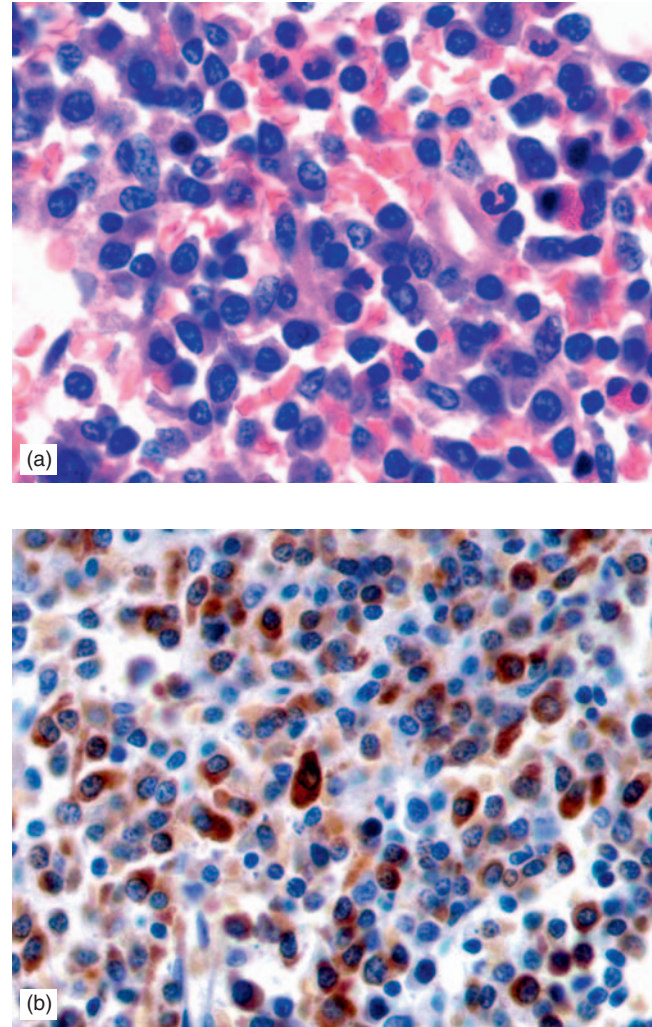
*Adapted from Ref. [133].

organ transplant population are of recipient origin [140]. This may suggest the escape of host EBV-infected cells from the regulatory mechanisms of the immune system. An EBV-associated protein, LMP-1, is engaged in signaling host proteins from the tumor necrosis factor (TNF) receptor family, leading to cell growth and transformation [141]. Unlike PTLD in solid organ transplants, the overwhelming majority of the PTLD cases in the bone marrow allograft recipients are of donor origin [142].

The proliferating B-cells in PTLD are BCL-6⁺ and MUM1⁺, consistent with a post-germinal center stage of B-cell differentiation [143]. Molecular studies have demonstrated amplification of *PAX5* at chromosome 9p13 region suggesting that this gene may play a role in the pathogenesis of PTLD [144].

Pathology

Early lesions consist of plasmacytic hyperplasia and IM-like disorders (Figure 19.10). These lesions mainly occur in oropharynx and lymph nodes and are characterized by preservation of the nodal sinuses and residual reactive follicles with diffuse interfollicular proliferation of plasma

**FIGURE 19.10** An early lesion of post-transplant lymphoproliferative disorder showing plasmacytic hyperplasia: (a) H&E and (b) CD138 stains.

cells and B-immunoblasts mixed with T-cells [133, 138]. Immunoblasts are commonly positive for EBV-LMP [145].

Polymorphic PTLD leads to the effacement of nodal architecture and/or destructive extranodal tissues [133]. The lymphoid infiltrates are polymorphic and consist of a mixture of small to large cells, including plasma cells and immunoblasts, mimicking mixed small and large cell lymphoma (Figure 19.11a) [133]. Scattered atypical large cells and necrotic areas may be present [133]. Some cases may show frequent mitosis. Immunophenotypic studies reveal a mixture of B- and T-cells, but B-cells may express monotypic or polytypic Ig light chains. The immunoblasts often express EBV-LMP and EBNA [133]. Molecular studies often show Ig gene rearrangement and/or EBV genome, frequently of type A [133, 146]. These lesions usually lack mutations in oncogenes and tumor suppressor genes such as *MYC*, *RAS*, and *TP53* [147].

Monomorphic PTLD demonstrates significant architectural alteration, monomorphic features, and cellular atypia consistent with the diagnosis of lymphoma (Figure 19.11b). These lesions are mostly of B-cell origin, but the

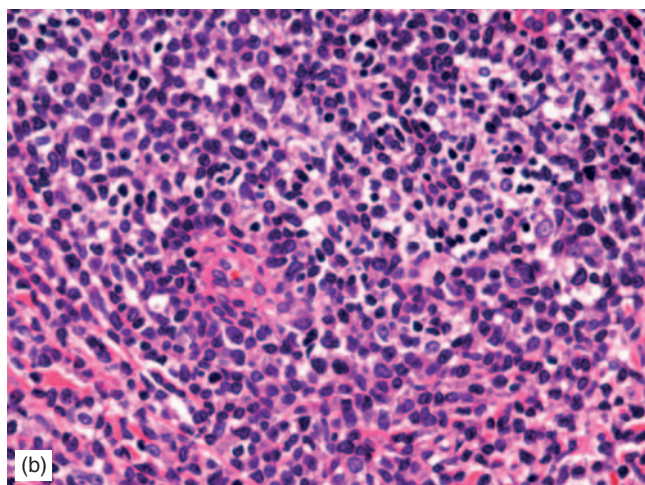
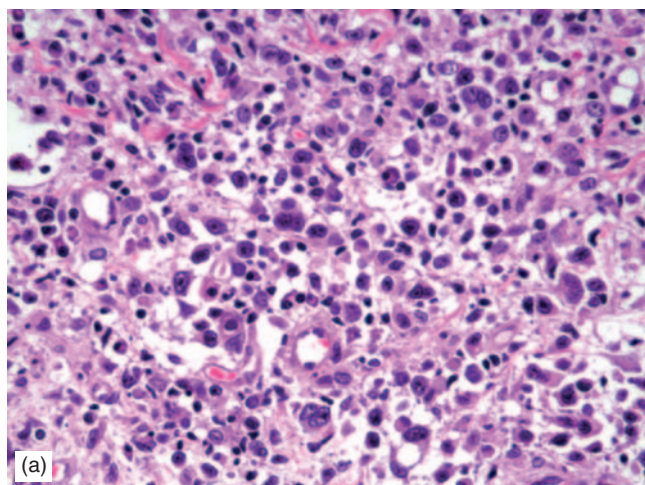


FIGURE 19.11 Post-transplant lymphoproliferative disorder. Lymph node biopsy sections demonstrating an example of polymorphic (a) and monomorphic (b) PTLD.

T-cell variants have also been reported in 4–14% of the cases [139, 148]. Monomorphic PTLD should be classified according to the WHO guidelines for the classification of B- and T-cell lymphomas (see Chapters 15–18). In some cases, the affected organs may show both polymorphic and monomorphic infiltrates in the same tissue section, and at the molecular level, they often evolve from polyclonal through oligoclonal and finally to monoclonal when a particular clone achieves predominance (Figure 19.12) [147].

Hodgkin lymphoma (HL) and HL-like PTLD are characterized by the presence of Hodgkin and Reed–Sternberg (HRS) cells or HRS-like cells. The classical HRS cells typically express CD15 and CD30 and lack CD45 expression, whereas HRS-like cells show atypical immunophenotypic features [149, 150]. The HRS-like cells are commonly EBV-positive.

Clinical Aspects

The major risk factors for the development of PTLD are immunosuppression and EBV infection. The more severe

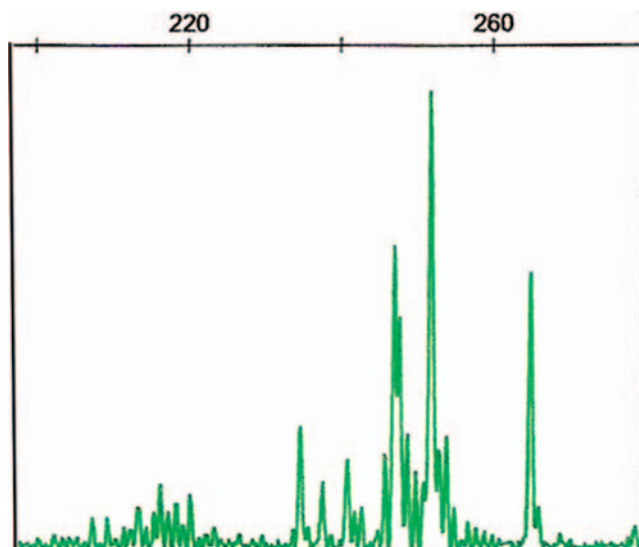


FIGURE 19.12 An example of oligoclonal post-transplant lymphoproliferative disorder by PCR analysis. The number of clonal peaks (IgM heavy chain) is too great to be considered monoclonal, but they are discrete enough to represent several clonal subsets (oligoclonal proliferation).

the immunosuppression, the greater the risk for PTLD [151–153]. The incidence of PTLD is significantly higher in the EBV-seronegative patients than in the EBV-seropositive ones, because of the higher risk of EBV infection in recipients who have no pre-transplant immunity to EBV [154, 155].

Other risk factors include age of recipient under the age of 25 years, fewer HLA matches, and history of pre-transplant malignancy [156, 157]. The risk of PTLD is highest in the first year of the post-transplant period [158].

The incidence of PTLD in solid organ transplants is high in intestinal and multiorgan transplants, ranging from 11% to 33% followed by 2% to 9% for lung, 2% to 6% for heart, 1% to 3% for kidney, and 1% to 2% for liver transplantations [152–154, 159]. The incidence of PTLD in bone marrow allograft recipients is about 1%, except for those who receive HLA-mismatched or T-cell-depleted bone marrow or are treated by immunosuppressive drugs for graft versus host disease. In this group of patients, the risk of PTLD is up to 20% [158].

Patients typically demonstrate local or generalized lymphadenopathy, sometimes with graft dysfunction or other organ failures due to extranodal lymphoid infiltrate [160]. EBV-negative and late-occurring cases have a higher tendency to be monomorphic [139, 148].

The therapeutic approaches include reduction in immunosuppression, antiviral therapy, and chemotherapy and/or radiation therapy (for the treatment of monotypic PTLD and HL) [161, 162]. A significant proportion of early PTLD lesions and polymorphic forms may regress by the reduction of immunosuppression [163]. The overall prognosis is poor, particularly in monomorphic PTLD [157, 164].

Differential Diagnosis

The differential diagnosis between different categories of PTLD, particularly between polymorphic and monomorphic

variants, is often difficult. The major morphologic, immunophenotypic, and molecular genetic differences between the PTLD subtypes are presented in Table 19.8.

Methotrexate-Associated Lymphoproliferative Disorder

Methotrexate-associated lymphoproliferative disorders are rare conditions seen in patients with autoimmune disorders (such as rheumatoid arthritis, psoriasis) treated with methotrexate. The lymphoproliferation may mimic polymorphous PTLD (about 14% of the cases) or a garden variety of lymphomas [133, 165, 166] (see Chapters 15–17). Partial or total regression of the lesion is seen in up to 60% of the cases in response to withdrawal of methotrexate therapy [133, 167, 168].

Lymphomatoid Granulomatosis

Lymphomatoid granulomatosis is an extranodal, angio-centric lymphoproliferative disorder consisting of large, EBV-positive B-cells in a background of polymorphous reactive cells, predominantly T-lymphocytes. It appears to be an EBV-induced disorder in an immunodeficiency setting, such as HIV infection, XLP syndrome, methotrexate therapy, allogeneic organ transplantations, or WAS [133, 169–171].

Morphology: Lymphomatoid granulomatosis is an angiocentric and angiodestructive polymorphous lymphoproliferative process characterized by the presence of a small number of large B-cells in a background of inflammatory cells. The inflammatory component is polymorphic consisting of a mixture of lymphocytes, plasma cells, immunoblasts, and histiocytes with scattered eosinophils and rare neutrophils (Figure 19.13). The large EBV-positive cells resemble immunoblasts, but bizarre large cells with multilobated nucleus or Reed–Sternberg-like cells may be present. This lymphoproliferative disorder characteristically infiltrates into the vascular structures and may cause vascular damage, fibrinoid necrosis, and ischemic changes in the surrounding tissues. There are three histological grades [172–174].

Grade I	Polymorphous lymphoid infiltrate with absent or rare large, immunoblastic, EBV-positive lymphocytes (>5/hpf).
Grade II	Polymorphous lymphoid infiltrate with moderate numbers of large, immunoblastic, EBV-positive lymphocytes (5–20/hpf). Necrosis is often present.
Grade III	Is considered a variant of DLBCL and consists of numerous large, immunoblastic, and EBV-positive lymphocytes (5–20/hpf). In some areas, these cells may appear in clusters or small sheets. Necrosis is often extensive. Large bizarre cells with multilobed nucleus and Reed–Sternberg-like cells are often present.

Immunophenotype: The large immunoblast-like cells are usually positive for CD20, CD45, and LMP1 [133]. The expression of CD79a and CD30 is variable, and CD15 is negative. The background lymphocyte population consists primarily of CD3+ T-cells. The CD4:CD8 ratio is often elevated.

Cytogenetic and molecular studies: No recurrent chromosomal abnormalities have been reported. There is evidence of clonal Ig gene rearrangement in most grade II and III cases. The clonal population may not be identical in different sites. Establishment of clonality in the grade I lesions may not be conclusive. Most of the cases show EBV infection demonstrated by EBER or other molecular markers. The EBV infection in some cases is clonal.

Clinical aspects: Lymphomatoid granulomatosis is a rare condition observed in immunodeficiency states, more often in adults and males [133, 172–174]. The most common sites of involvement in order of frequency are lung, skin, kidney, liver, and brain. Other sites such as upper respiratory and gastrointestinal tracts, spleen, and lymph nodes are occasionally affected. Clinical symptoms are related to the involved organ, with respiratory symptoms being the most common presentation. The clinical course, particularly for the grades II and III, is often aggressive. However, some patients may show a fluctuating clinical course with occasional spontaneous remission.

Senile EBV-Associated B-Cell Lymphoproliferative Disorder

Senile EBV-associated B-cell lymphoproliferative disorder is a recently described entity with a high incidence in elderly people (>60 years old) and without underlying immunodeficiencies [175, 176]. It is characterized by an EBV-positive B-cell proliferation and a polymorphic cellular composition consisting of varying numbers of centroblasts, immunoblasts, and HRS-like cells [175, 177]. Angiocentric growth pattern and areas of necrosis are often present. This condition involves both lymph nodes and extranodal tissues. Two morphologic subtypes have been described: (1) polymorphic type with a relatively favorable clinical course and (2) large cell lymphoma type with aggressive clinical course. The EBV+ HRS-like cells in this disorder are positive for CD20 and CD45. They may variably express CD30 but are negative for CD15 [176].

Lymphomatoid Papulosis

Lymphomatoid papulosis is a benign, chronic recurrent skin disorder which occurs at all ages, but the peak incidence is around 50 years of age [178, 179]. The male:female ratio is about 2:1. It is characterized by multiple spontaneously regressing papules consisting of a polymorphic infiltrate including anaplastic large lymphocytes and cells resembling HRS cells (Figure 19.14). These cells represent activated CD4+ cells and coexpress CD30 but are negative for anaplastic lymphoma kinase. In some cases, lymphomatoid papulosis after a long period (~15 years) may evolve into anaplastic large cell lymphoma, Hodgkin lymphoma, mycosis fungoides, or other T-cell malignancies [180–183].

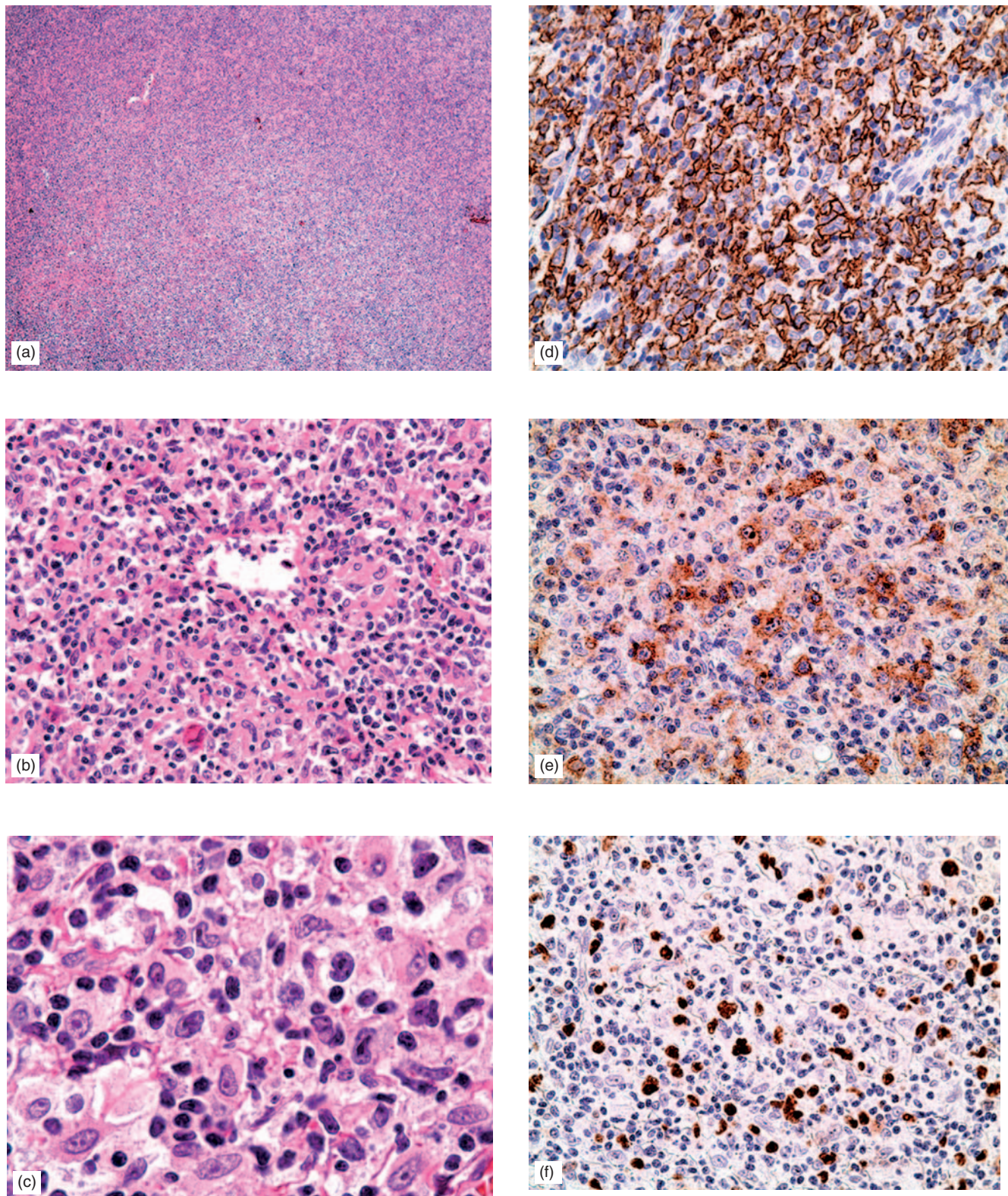


FIGURE 19.13 Lymphomatoid granulomatosis. Lung biopsy section demonstrates a dense polymorphic, angiocentric lymphoid infiltrate: (a) low power, (b) intermediate power, and (c) high power views. Numerous large cells are CD20-positive (d) and some express CD30 (e) and/or EBV-EBER (f). Courtesy of Sophie Song, MD, PhD, Department of Pathology and Laboratory Medicine, UCLA Medical Center.

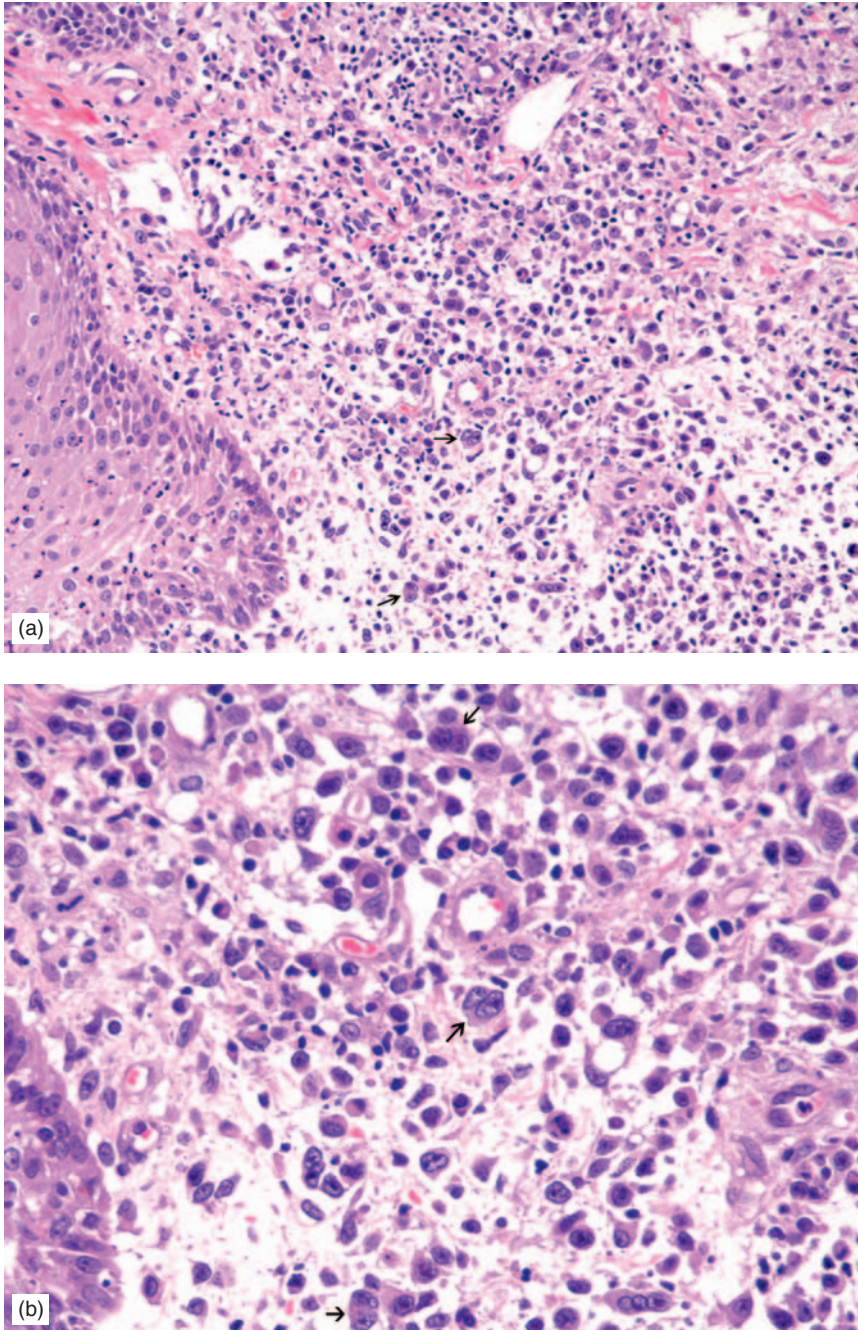


FIGURE 19.14 Lymphomatoid papulosis. Skin biopsy demonstrates a polymorphous infiltrate with atypical large lymphocytes and Reed–Sternberg-like cells: (a) low power and (b) high power views.

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Mastocytosis

Faramarz Naeim

Mastocytosis refers to an abnormal proliferation of mast cells in one or multiple organs. It covers a wide spectrum of clinicopathologic disorders from localized to systemic and from indolent to aggressive forms. Most systemic variants of mastocytosis are the result of clonal expansion of mast cells [1–3].

Mast cells are derived from the hematopoietic stem cells. The committed mast cell progenitors express CD33, CD34, and CD117 (*c-kit*) and are detectable in the bone marrow and peripheral blood [4, 5]. Mast cells are distinguished from other granulocytic cells by their unique phenotypic and functional properties (Tables 20.1 and 20.2). Mast cells produce a substantial amount of histamine and heparin and express surface IgE receptor [6–8]. In contrast to basophils and other granulocytic cells, mast cells have a significantly longer *in vivo* life span ranging from several months to years [7, 8].

ETIOLOGY AND PATHOGENESIS

The etiology and pathogenesis of mastocytosis are not clearly understood. The *c-kit* protooncogene encodes KIT, a tyrosine kinase receptor, which binds stem cell factor (SCF) [5, 9]. Systemic mastocytosis (SM) has been associated with somatic *c-kit* mutation at codon 816, substituting valine to aspartate (Figure 20.1) [2, 3, 10, 11]. Activated mutated *c-kit* along with increased production of SCF may play a role in the pathogenesis of SM [12, 13]. Genetic factors appear to play an important role in the childhood mastocytosis based on the report of 25% of congenital mastocytosis in pediatric cases and concordant symptoms of mastocytosis observed in 10 pairs of homozygotic twins [14, 15].

PATHOLOGY

Morphology

Mastocytosis is demonstrated as multifocal clusters or diffuse infiltration of mast cells in the skin, bone marrow, spleen, liver, gastrointestinal tract, and other tissues [16–18]. Mast cells in smears stained with Wright's or Giemsa stains are very distinct and appear as medium- to large-sized cells with abundant cytoplasm loaded with small deeply basophilic granules, often masking the nucleus (Figure 20.2). The nuclei are round, oval, or spindle-shaped and show a dense chromatin [16]. Some mast cells may be hypogranular or appear immature. Binucleated or multinucleated mast cells may be present [16].

Mast cells in the H&E-stained sections appear as medium-sized cells with variable amounts of granular cytoplasm and round, oval, or spindle-shaped nucleus with condensed chromatin (Figure 20.3). The cytoplasmic granules are faintly eosinophilic in the H&E sections and variable in amount. The granules in some mast cells are sparse and difficult to detect. The hypogranular mast cells may demonstrate an abundant pale cytoplasm resembling histiocytes, monocytoïd B-cells, or hairy cells [1]. The spindle-shaped mast cells may mimic fibroblasts. Because of these overlapping morphologic features, it is highly recommended to perform additional accessory studies such as cytochemical stains, immunophenotyping, and molecular analysis to establish the diagnosis of mastocytosis (Table 20.3). Mast cell infiltration is often associated with various degrees of fibrosis and the presence of inflammatory cells, such as lymphocytes and eosinophils (Figures 20.4 and 20.5).

TABLE 20.1 Immunophenotypic features of mast cells.*

CD	Mast cells			
	Normal	Neoplastic	Basophils	Monocytes
CD2	—	+	—	—
CD13	—	±	+	+
CD14	—	—	—	+
CD15	—	—	—	+
CD25	—	+	+	±
CD33	+	+	+	+
CD34	—	—	—	—
CD45	+	+	+	+
CD117	+	+	—	—

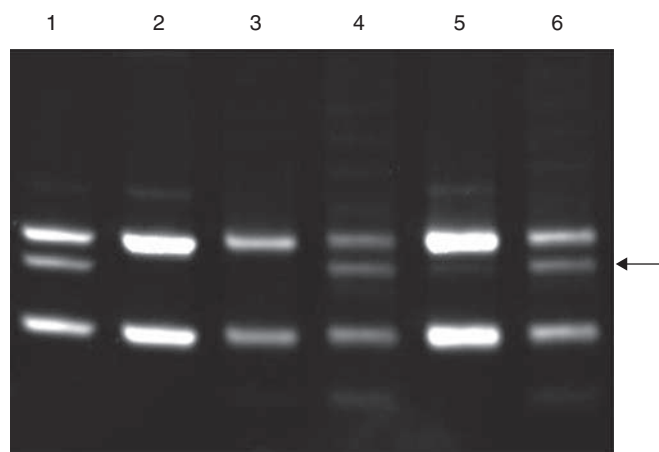
*Adapted from Ref. [17].

TABLE 20.2 Major mast-cell-derived mediators and their effects in systemic mastocytosis.*

Mediators	Clinicopathological effects
Histamine	Vascular instability, urticaria, headache, edema, flushing, gastric hypersecretion, abdominal pain, bronchial constriction, diarrhea
Heparin	Coagulation abnormalities, bleeding
Tryptases	Fibrosis, angiogenesis, tissue remodeling, degradation of matrix molecules, bone resorption
tPA	Fibrinolysis
VEGF	Increased angiogenesis, edema
PGD2	Edema, urticaria, flushing, bronchial constriction
bFGF	Fibrosis, osteosclerosis, angiogenesis
TNF- α	Activation of endothelial cells, vascular instability
TNF- β	Fibrosis, abnormal bone remodeling, osteopenia
Interleukins (IL-1, -2, -3, -5, -6, -9, -10, -11, -13, GM-CSF)	Eosinophilia, bone marrow lymphocytosis, myeloid hyperplasia, activation of stromal cells, fibrosis
Chemokines (MCP-1, MIP-1 α , others)	Leukocyte activation, accumulation of lymphocytes, monocytes, and eosinophils

*Adapted from Ref. [17].

The WHO criteria for the diagnosis of mastocytosis are presented in Table 20.3. For the diagnosis of SM, the major criteria are the presence of multifocal infiltrate of mast cells in bone marrow biopsy sections or other extracutaneous sites, confirmed by tryptase immunohistochemistry or other special stains [1]. Minor criteria include (1) the presence of 25% of mast cells being spindle-shaped, atypical, or immature,

**FIGURE 20.1** Demonstration of D816V *C-KIT* mutation by restriction fragment length polymorphism. Lane 1 is a positive control. Patient samples in lanes 4 and 6 show the presence of an additional band (arrow) as a result of a new restriction site by an A→T nucleotide change in *c-kit* codon 816. From Ref. [10] by permission.

(2) detection of *KIT* mutation, (3) co-expression of CD117, CD2, and CD25 by the infiltrating mast cells, and (4) serum tryptase levels of >20 ng/mL. Diagnosis of SM could be made when one major and one minor criteria or three minor criteria are present.

Skin and bone marrow biopsies are the most frequent tissue samples obtained for the diagnosis of cutaneous and systemic mastocytosis, respectively. In cutaneous mastocytosis (CM), aggregates or sheets of elongated or spindle-shaped mast cells are present in the papillary and/or reticular dermis. These infiltrates are often around vascular structures [1, 16].

The bone marrow biopsy sections often show multiple mast cell aggregates. These aggregates may be either paratrabecular or interstitial (Figures 20.3–20.5). Paratrabecular infiltrates may show extensive fibrosis and osteosclerosis with scattered or aggregates of spindle-shaped mast cells which are identified by the Giemsa stain or immunohistochemical stains for tryptase or CD117 (Figures 20.6 and 20.7). Mastocytosis may be a part of a clonal primary hematopoietic disorder, such as myelodysplastic syndrome, chronic myeloproliferative disorder, or acute myelogenous leukemia.

In mast cell leukemia, mast cells account for $\geq 20\%$ of the bone marrow nucleated cells, often with the presence of circulating mast cells in the peripheral blood. The bone marrow differential count on the smears should be performed in the areas away from bone marrow tissue particles [17].

Splenic involvement in mastocytosis usually consists of infiltrating mast cells randomly distributed in the red pulp or appearing in aggregates adjacent to the white pulps or trabeculae [1, 19]. The mast cell clusters are often associated with variable amounts of fibrosis and are mixed or surrounded by lymphocytes, plasma cells, and/or eosinophils. In the liver, mast cells are found in the sinuses and/or portal areas with focal areas of fibrosis (Figure 20.8). The infiltrating mast cells are detected by special stains, such as Giemsa, or immunophenotypic studies (discussed later).

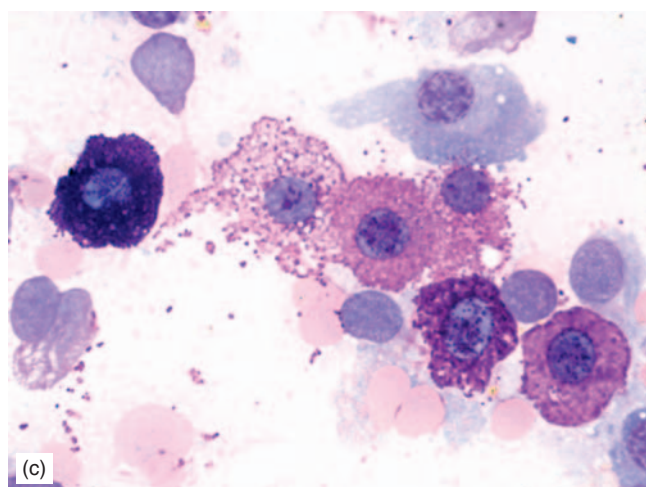
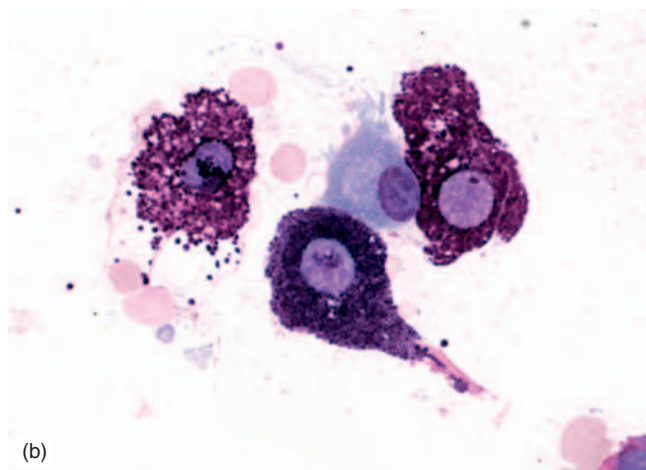
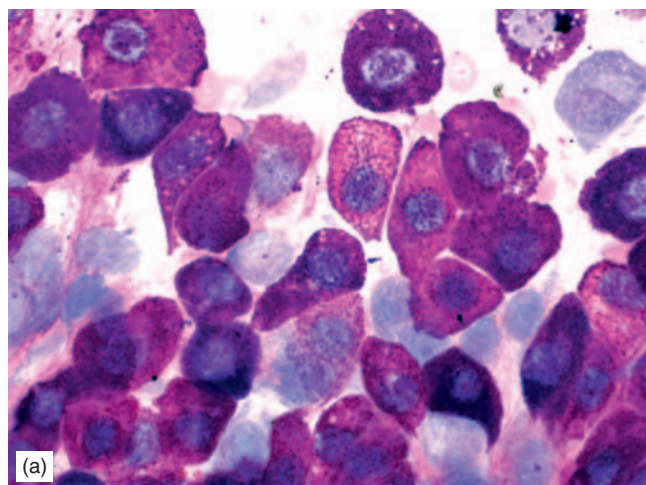


FIGURE 20.2 Mast cells. Wright-stained bone marrow smears demonstrating mast cells with abundant cytoplasm containing variable amounts of small, deeply basophilic granules (a, b, and c).

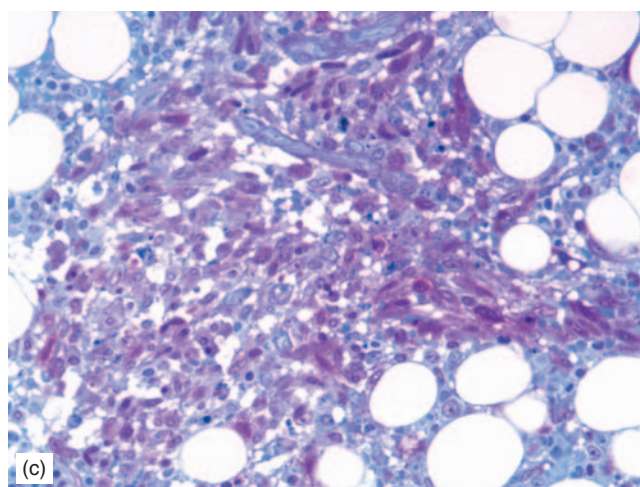
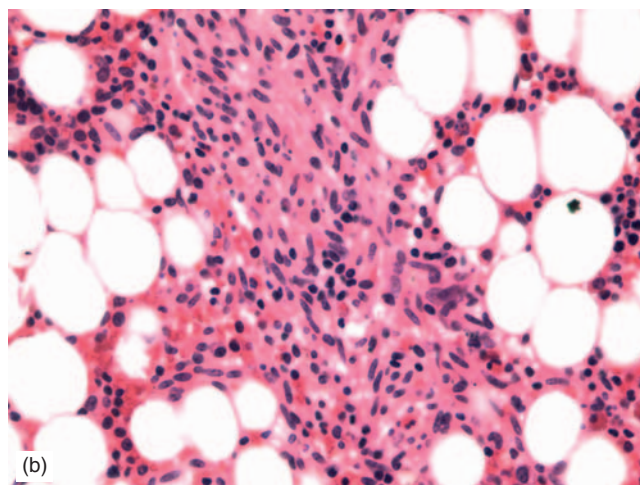
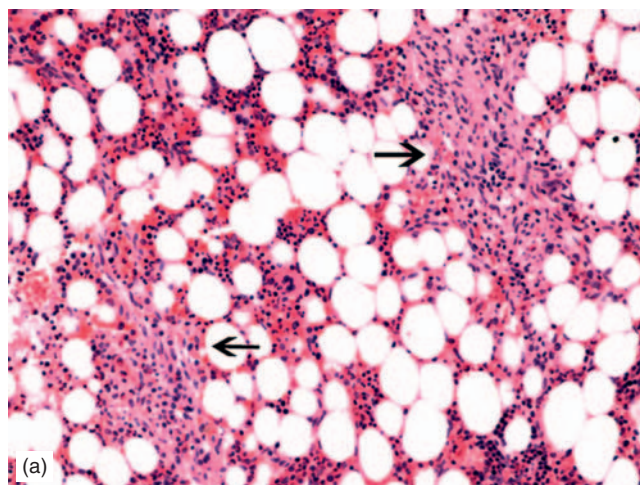


FIGURE 20.3 Systemic mastocytosis. Bone marrow biopsy section demonstrating cluster of mast cells resembling histiocytes or fibroblasts: (a) low power view, arrows, and (b) high power view. Mast cells are often easily identified by Giemsa stain (c).

TABLE 20.3 WHO criteria for the diagnosis of cutaneous and systemic mastocytosis.*

Cutaneous mastocytosis
Mast cell infiltrates in a multifocal or diffuse pattern in the skin biopsies with typical clinical findings.
Systemic mastocytosis
<i>Major criterion</i>
Multifocal infiltrates of mast cells (≥15 mast cells in each aggregate) in one or more extracutaneous sites confirmed by tryptase immunohistochemistry or other special stains.
<i>Minor criteria</i>
1. More than 25% of mast cells are spindle-shaped or atypical in the extracutaneous infiltrates in the biopsy sections and/or smear preparations.
2. Detection of <i>KIT</i> mutation at codon 816.
3. Co-expression of CD117, CD2, and/or CD25 by the infiltrating mast cells.
4. Persistent total serum tryptase levels >20 ng/mL in cases not associated with clonal myeloid disorders.

*Adapted from Ref. [1].

Immunophenotype

Normal mast cells express CD33, CD45, CD68, CD117, and tryptase and are negative for CD14, CD15, CD16, and MPO (Figures 20.4, 20.5, and 20.7). The neoplastic mast cells show aberrant expression of CD2 and CD25 [1, 17, 20–24].

Molecular and Cytogenetic Studies

SM has been associated with somatic *c-kit* mutations. The most common mutation is reported at codon 816, substituting valine to aspartate. This can be detected by RFLP or DNA sequence analysis (Figures 20.1 and 20.9) [2, 3, 10, 11]. A report in gene expression analysis in mastocytosis demonstrated significant upregulation of genes for α-tryptase, the activating transcription factor type 3, and the muscle aponeurotic fibrosarcoma type F as surrogate markers, strongly correlated with serum tryptase levels [25].

CLINICAL ASPECTS

Mast cell diseases are divided into two major clinical entities: (1) cutaneous mastocytosis and (2) systemic mastocytosis [1, 8, 26].

Cutaneous Mastocytosis

Cutaneous mastocytosis consists of mast cell disorders limited to skin without evidence of systemic involvement, such as elevated levels of serum tryptase, bone marrow

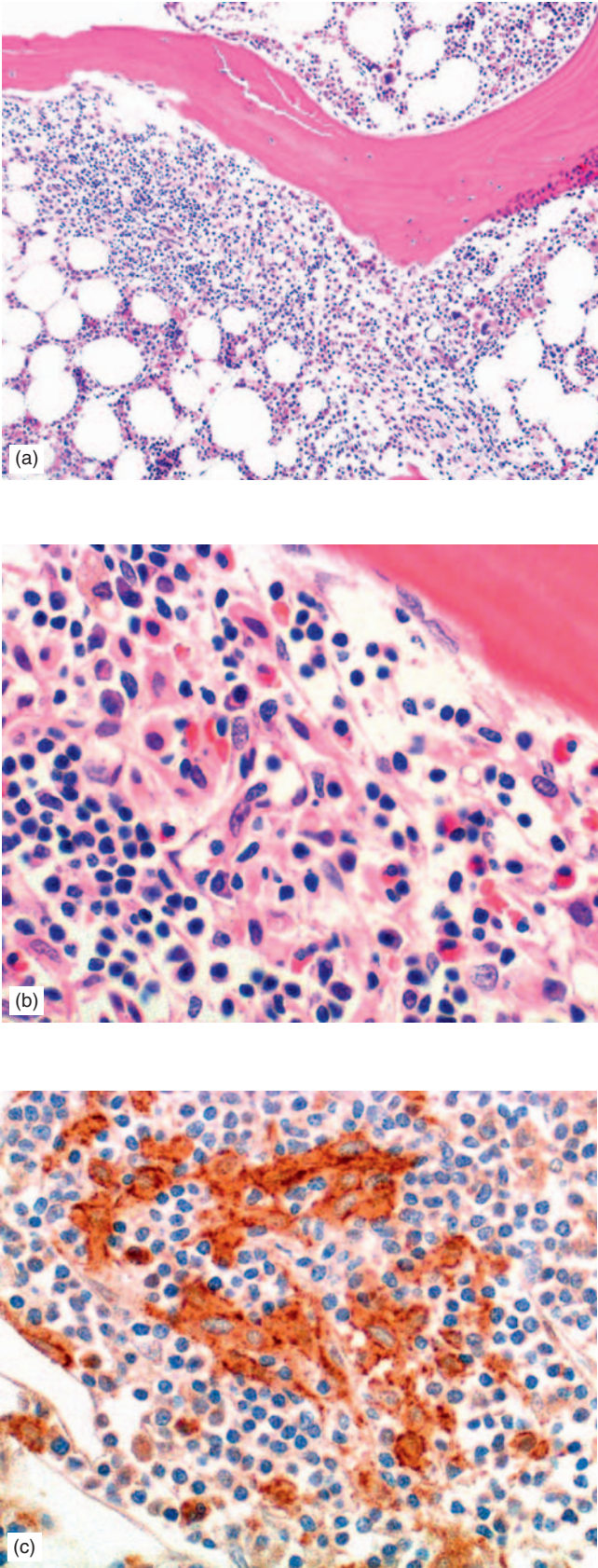


FIGURE 20.4 A cluster of mast cells mixed with lymphocytes and histiocytes is present next to bone: (a) low power and (b) high power views. Mast cells show expression of CD117 by immunohistochemical technique (c).

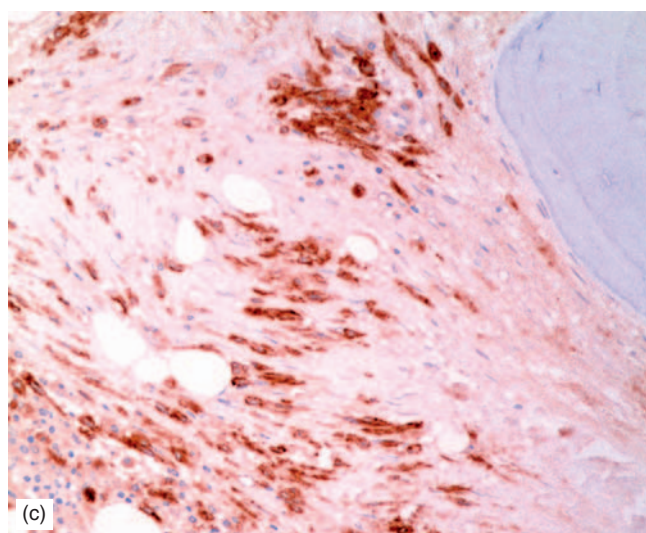
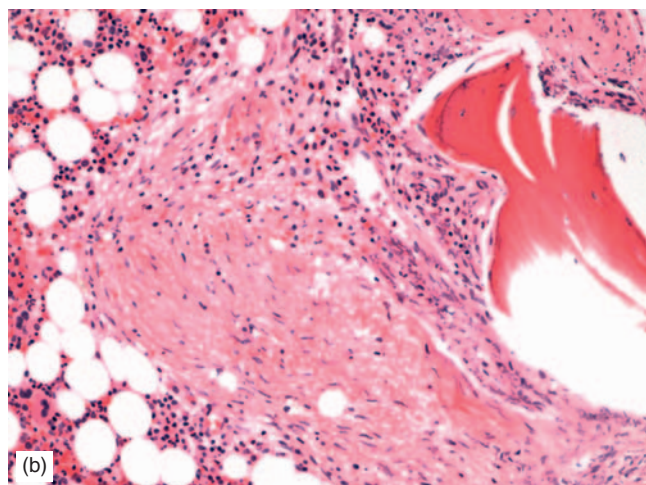
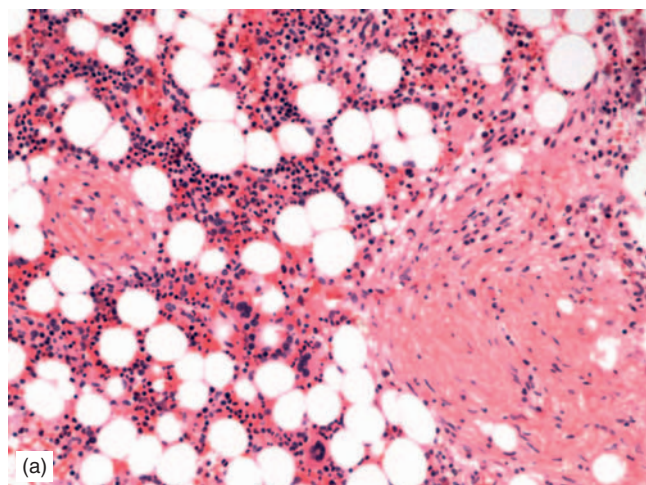


FIGURE 20.5 Bone marrow biopsy section demonstrating areas of dense fibrosis: (a) low power and (b) high power views. Clusters of CD117-positive-elongated cells representing mast cells are present (c).

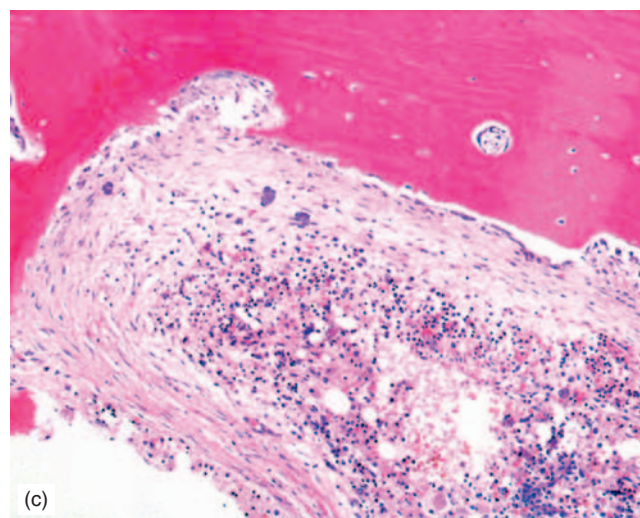
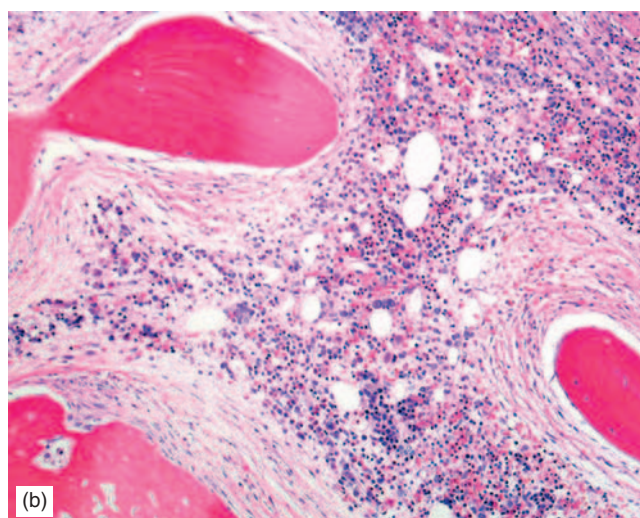
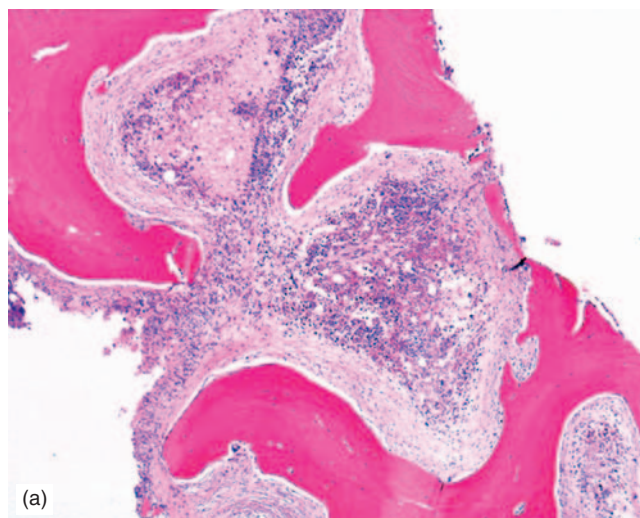


FIGURE 20.6 Bone marrow involvement in systemic mastocytosis. Extensive paratrabecular fibrosis and some osteosclerosis are present: (a) low power, (b) intermediate power, and (c) high power views.

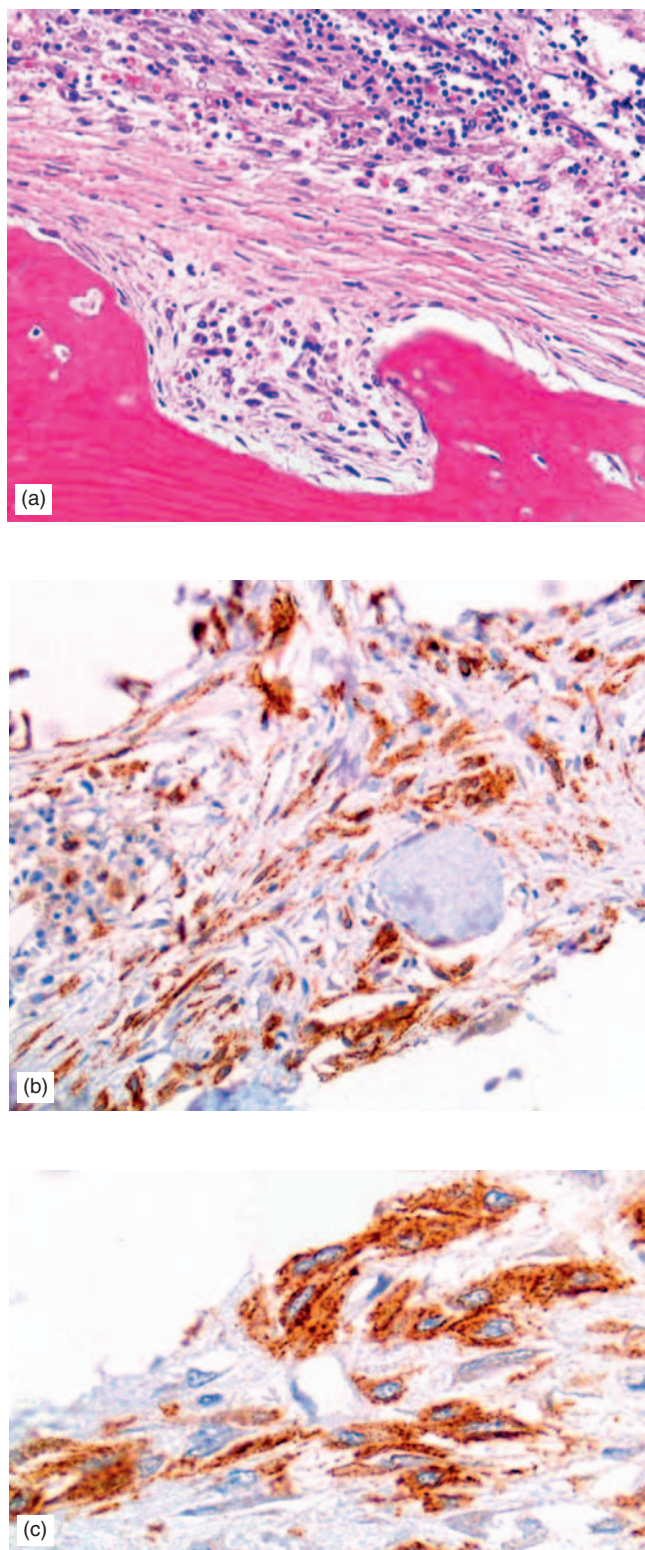


FIGURE 20.7 Systemic mastocytosis. Bone marrow biopsy section showing paratrabecluar fibrosis (a) with the presence of numerous tryptase-positive cells in the fibrotic area: (b) low power and (c) high power views.

infiltration, or organomegaly. There are four major clinico-pathologic subtypes: (1) urticaria pigmentosa, (2) cutaneous mastocytoma, (3) diffuse CM, and (4) telangiectasia macularis eruptiva perstans. The diagnosis is made based on the clinical presentation and by a skin biopsy demonstrating significant increase in mast cells (usually ≥ 20 mast cells per high power field), which are particularly found around vascular structures [9, 27, 28].

Urticaria pigmentosa (UP) is the most common mast cell disorder in children and adults. The *KIT* point mutation in the pediatric and adult UP appears to be different from the mutation of codon 816 observed in SM. There are reports of mutations in codon 839 and codon 516 in pediatric and adult UP, respectively [3, 29].

The cutaneous lesions are usually small yellow-tan to reddish-brown macules or papules. Plaque-like lesions may also occur [9]. The upper and lower extremities are the most frequently affected sites. The face, palms, and soles are not involved. Most of the affected children are under the age of 1 and rarely show systemic involvement. The UP-associated pruritis is exacerbated by a variety of stimulants, such as change in temperature, spicy food, or local friction [9, 29].

Cutaneous mastocytoma is typically a solitary lesion occurring in early childhood, usually before 6 months of age [9, 30, 31]. The trunk and wrist are frequent sites of involvement [1]. Large clusters or sheets of mast cells are present in the papillary and reticular dermis.

Diffuse cutaneous mastocytosis is a childhood disorder usually occurring before the age of 3. The skin is diffusely infiltrated but relatively smooth. It may show increased thickness and/or a yellowish-brown color. The maculopapular lesions are usually absent [9, 27, 32].

Telangiectasia macularis eruptiva perstans is a rare cutaneous mast cell disorder mainly occurring in adults. It is characterized by tan-brown macules with telangiectasia but no blisters or pruritis [9, 33].

Systemic Mastocytosis

Systemic mastocytosis represents mast cell disease beyond skin. The increased mast cells are found in extracutaneous sites with or without skin involvements. The frequently involved extracutaneous sites include bone marrow, liver, spleen, lymph nodes, and gastrointestinal tract [1, 9, 34, 35a]. The WHO requirements for the diagnosis and classification of SM are demonstrated in Tables 20.3 and 20.4 [1].

Clinical manifestations of mastocytosis are the result of two different mechanisms: (1) mediator release from mast cells and (2) growth and infiltration of the mast cells in various organs.

A wide variety of mediators are released from mast cells resulting in clinical symptoms, such as headache, flushing, pruritis, hypotension, and diarrhea (Table 20.2). The growth and infiltration of mast cells in various organs may lead to organomegaly as well as organ dysfunction, leading to ascites, cytopenia, malabsorption, and pathologic fractures. The organopathy-related clinical symptoms are referred to as *C-findings*, whereas organomegalies without any evidence of organopathy are termed *B-findings* [17, 18].

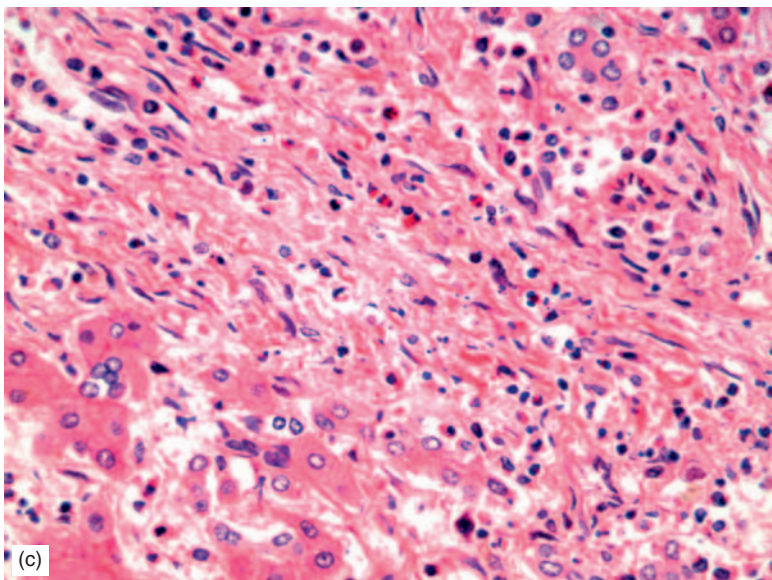
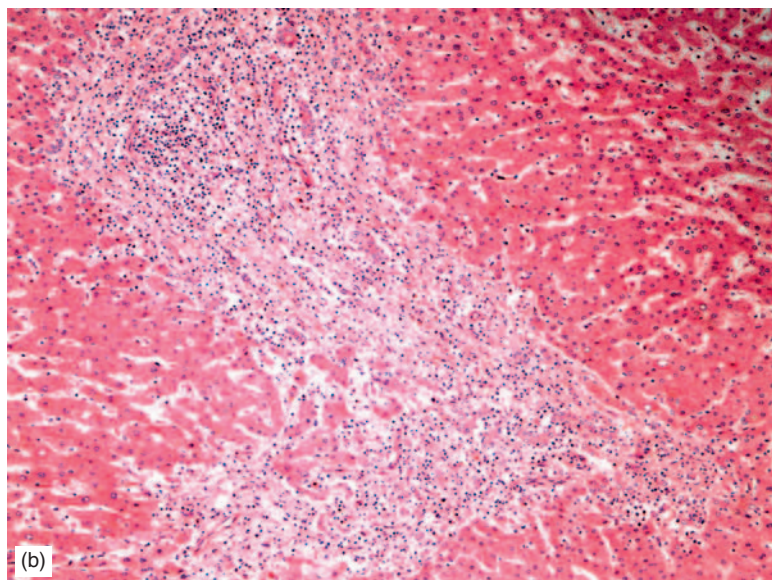
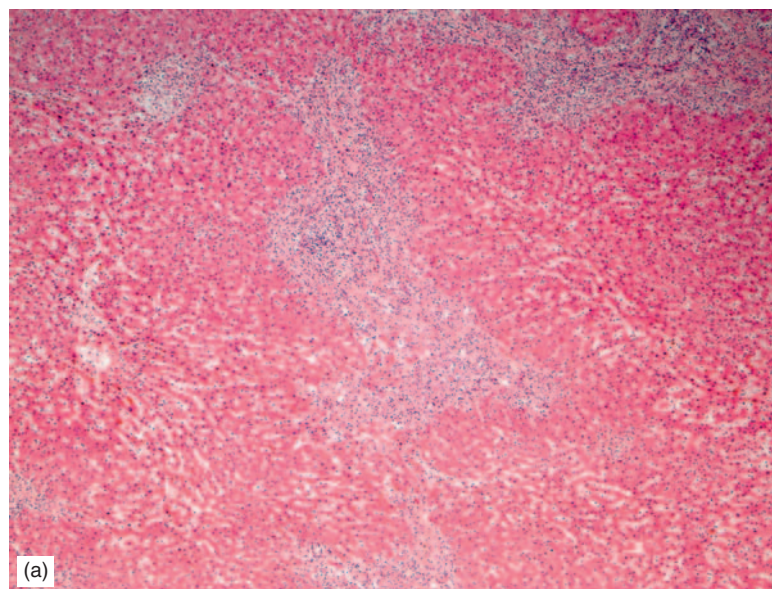


FIGURE 20.8 Mastocytosis involving the liver. Fibrosis and infiltration of the inflammatory cells are noted in the expanded portal areas: (a) low power and (b) intermediate power views. Cells with elongated or spindle-shaped nuclei represent mast cells: (c) high power view.

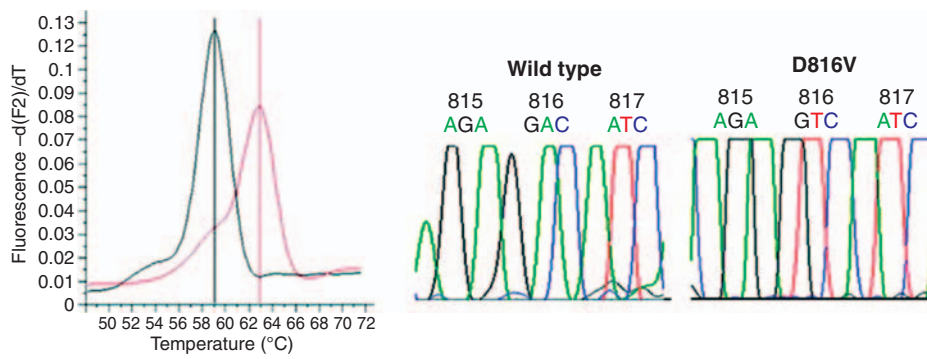


FIGURE 20.9 D816V *c-KIT* mutation in a patient with systemic mastocytosis. From Ref. [11] by permission. This research was originally published in *Blood*.

TABLE 20.4 WHO classification of systemic mastocytosis.*

Indolent systemic mastocytosis
Systemic mastocytosis with associated clonal, hematological non-mast cell lineage disease
Aggressive systemic mastocytosis
Extracutaneous mastocytoma
Mast cell sarcoma

*Adapted from Ref. [1].

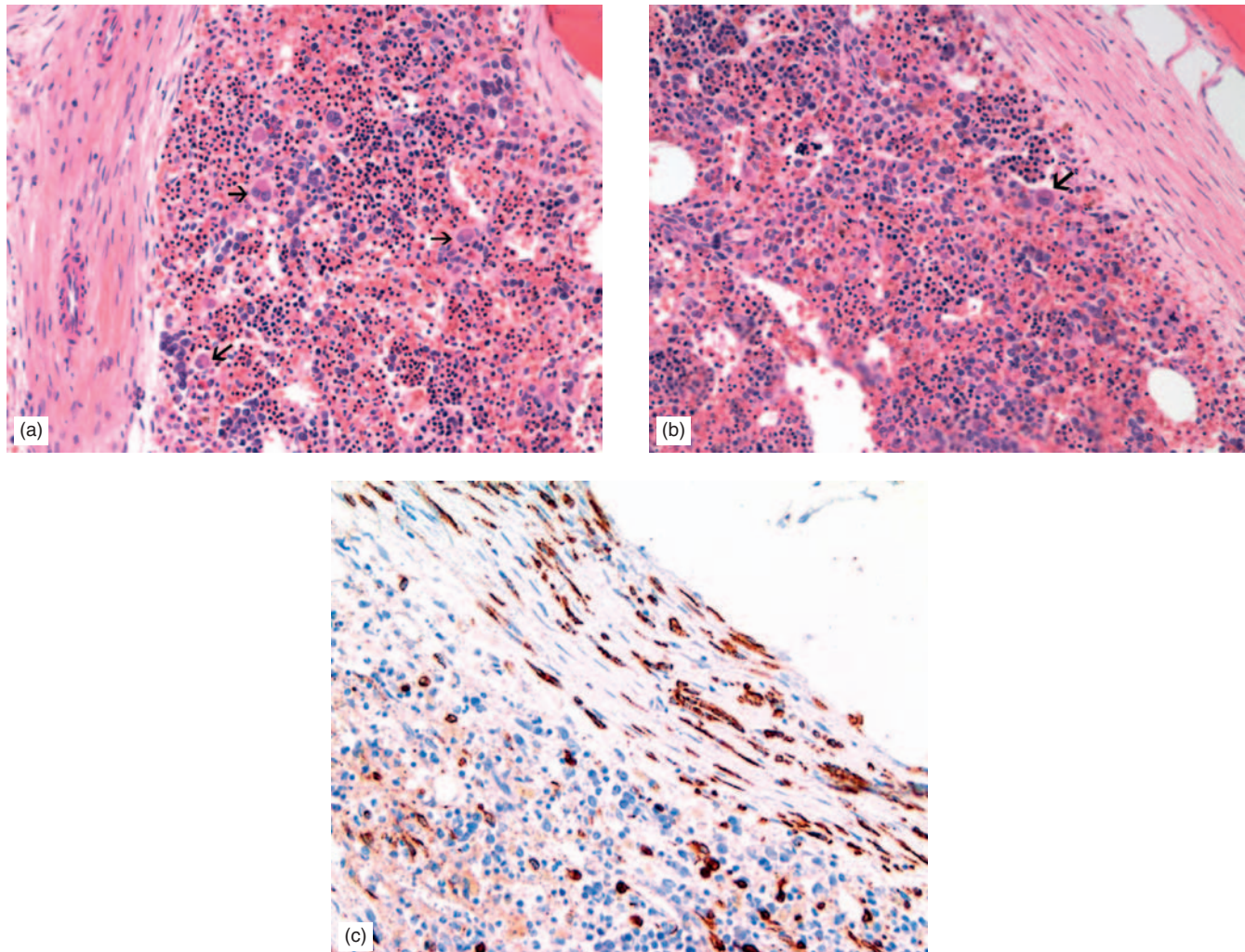


FIGURE 20.10 Mastocytosis in a patient with myelodysplastic syndrome. A biopsy section showing paratrabeular fibrosis. The bone marrow is hypercellular with the presence of numerous micromegakaryocytes (a and b; arrows). Numerous spindle cells are positive for CD117 in the fibrotic area (c).

TABLE 20.5 Comparison of B- and C-findings in subtypes of systemic mastocytosis.*

Findings	ISM			SM-AHNMD	ASM	MCL
	Typical ISM	BMM	SSM			
<i>B-findings</i>						
Hepatomegaly	—	—	±	±	±	±
Splenomegaly	—	—	+	±	+	±
Lymphadenopathy	—	—	±	±	±	±
Tryptase >200 ng/mL	—	—	+	±	±	±
<i>C-findings</i>						
Anemia (Hb <10g/dL)	—	—	—	±	+	+
Thrombocytopenia (<100 × 10 ⁹ /L)	—	—	—	±	+	+
Neutrophil count <1 × 10 ⁹ /L	—	—	—	±	+	+
Ascites or portal hypertension	—	—	—	—	+	+
Hypersplenism	—	—	—	±	+	±
Malabsorption with weight loss	—	—	—	—	±	±
Osteolysis	—	—	—	—	+	+
<i>Others</i>						
Urticaria pigmentosa-like lesions	+	—	±	±	±	—
Elevated serum LDH	—	—	—	±	±	+
Abnormal coagulation	—	—	±	±	±	+

*Adapted from Ref. [17].

ISM: indolent systemic mastocytosis, BMM: isolated bone marrow mastocytosis, SSM: smoldering systemic mastocytosis, SM-AHNMD: systemic mastocytosis with associated clonal hematological non-mast cell lineage disease, ASM: aggressive systemic mastocytosis, MCL: mast cell leukemia.

SM has been divided into the following categories (Table 20.4).

Indolent systemic mastocytosis (ISM) is referred to cases with relatively low burden of mast cells and therefore to an indolent clinical course and good prognosis. The majority of the patients with ISM have UP and show evidence of systemic involvement but lack C-findings. ISM accounts for >80% of all cases of SM [9]. Two subtypes of ISM have been described: smoldering systemic mastocytosis (SSM) and isolated bone marrow mastocytosis (BMM) [17]. In SSM, B-findings are present, and in BMM, there is lack of skin involvement (Table 20.5).

Systemic mastocytosis with associated clonal hematological non-mast cell lineage disease (SM-AHNMD) is mastocytosis associated with acute myeloid leukemias, acute lymphoid leukemias, myelodysplastic syndromes, chronic myeloproliferative disorders, or lymphoma (Figures 20.10 and 20.11).

While the *FIP1L1/PDGFR* fusion gene is mainly known for its association with hypereosinophilia (Chapter 9, p. 174 and Figure 9.20), this abnormal gene is also identified in patients with systemic mastocytosis. Eosinophilia is also frequently observed in systemic mastocytosis, and up to half of these cases are also positive for the *FIP1L1/PDGFR* fusion gene [35b].

Aggressive systemic mastocytosis (ASM) is characterized by the presence of organ-function impairment and

C-findings, leading to an aggressive clinical course [24, 36]. C-findings include (1) anemia, thrombocytopenia, and/or leukopenia, (2) hepatomegaly with ascites or portal hypertension, (3) splenomegaly with hypersplenism, (4) malabsorption and weight loss, and (5) osteolysis and pathologic fractures (Table 20.5).

Less than 50% of patients in this category show UP lesions. Mast cells account for <20% of the bone marrow nucleated cells. The *KIT* mutation in codon 816 is the typical molecular finding [36].

Mast cell leukemia is a rare condition characterized by diffuse infiltration of the bone marrow by atypical and/or immature mast cells. The pattern of bone marrow infiltration is usually interstitial. Mast cells comprise ≥20% of the nucleated cells in the bone marrow smears and ≥10% of the leukocyte differential counts in the peripheral blood (Figure 20.12) [1, 37–39]. Prognosis is extremely poor with an estimated survival of 6–12 months [9].

Extracutaneous mastocytoma is an extremely rare lesion consisting of an accumulation of mature mast cells in extracutaneous sites, such as the lung [40].

Mast cell sarcoma is another extremely rare lesion consisting of an infiltrating growth of atypical and/or immature mast cells with a potential of distant metastasis or progression to a leukemic phase [41, 42].

In general, the therapeutic approaches depend on the clinical symptoms and extent of the disease. Mediator-related

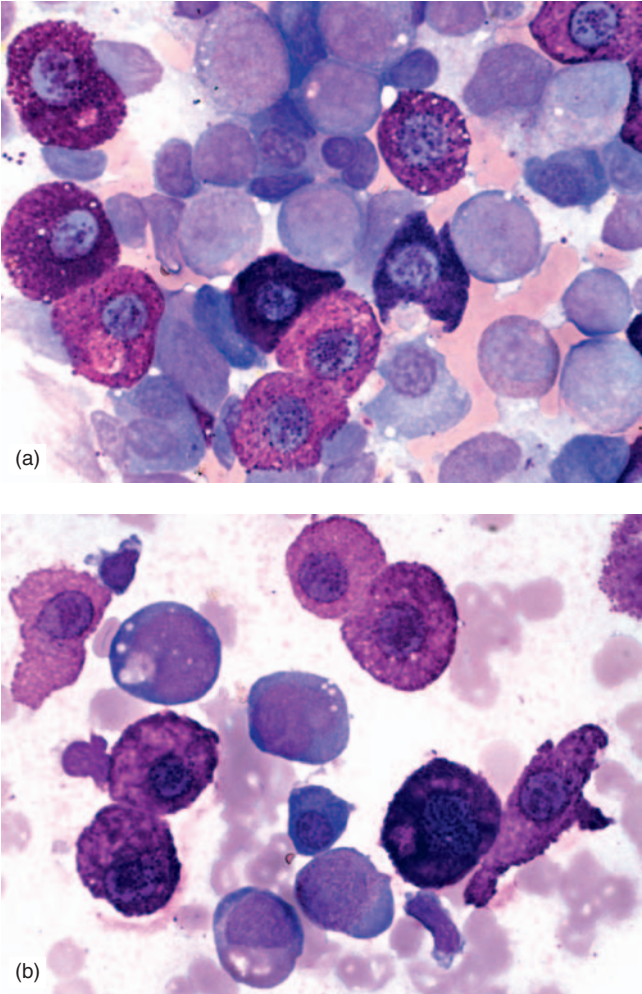


FIGURE 20.11 Mastocytosis in a patient with acute myelogenous leukemia. Bone marrow smear consisting of a mixture of myeloblasts and mast cells (a and b).

symptoms are treated with drugs that interfere with mediator production/release or mediator functions, such as histamine and leukotriene antagonists, glucocorticoids, cromolyn sodium, and aspirin [17, 26]. In addition to the anti-mediator drugs, patients with CM may receive psoralen and ultraviolet-A [43]. Cytoreductive drugs such as interferon- α , cytosine arabinoside, cladribine, vincristine, and doxorubicin are preserved for patients who have clear signs of aggressive disease [17, 44].

DIFFERENTIAL DIAGNOSIS

The differential diagnosis includes two major categories: (1) disorders with similar clinical manifestation but lack of histologic evidence of CM or SM and (2) disorders associated with increased mast cells or elevated serum tryptase (Table 20.6).

Disorders with similar clinical manifestation but lack of histologic evidence of CM or SM include anaphylaxis (may show elevated serum tryptase during, but not in the period between, the acute events), angioedema, carcinoid

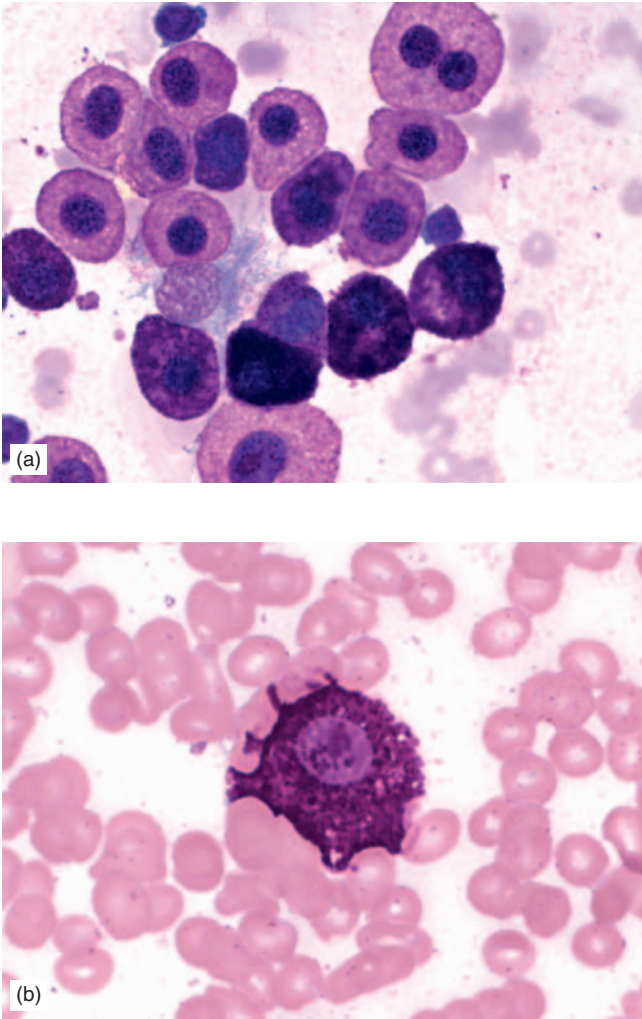


FIGURE 20.12 Bone marrow smear from a patient with mast cell leukemia demonstrating numerous mast cells (a). A mast cell is shown in peripheral blood smear (b).

TABLE 20.6 Differential diagnosis of mastocytosis.

Categories	Examples
Similar clinical manifestations but lack of histologic evidence of cutaneous or systemic mastocytosis	Anaphylaxis, angioderma, carcinoid syndrome, pheochromocytoma, Zollinger–Ellison syndrome
Associated with increased mast cells	Basal cell carcinoma, melanoma, lymphoma, helminth infection
Associated with elevated serum tryptase but lack of mastocytosis	Acute myelogenous leukemia, myelodysplastic syndrome, chronic myeloproliferative disorders
Morphologic overlap	Basophilic leukemia, histiocytic disorders, bone marrow metastasis, disorders associated with paratrabeular bone marrow fibrosis, such as chronic renal failure

syndrome, pheochromocytoma, and Zollinger–Ellison syndrome. All these disorders except acute episodes of anaphylaxis lack elevated serum tryptase or urinary histamine [9].

Disorders associated with increased mast cells or elevated serum tryptase consist of reactive conditions associated with mastocytosis or hematopoietic malignancies associated with elevated serum tryptase but lack of mastocytosis [44]. Reactive mast cell hyperplasia has been observed in various conditions, such as basal cell carcinoma, melanoma, helminth infection, and lymphomas. Elevated serum tryptase levels have been reported in a variety of clonal myeloid disorders, such as acute myelogenous leukemia, myelodysplastic syndromes, and chronic myeloproliferative disorders [8, 45]. Also, some patients with myelodysplastic syndrome may show *c-kit* mutation [46].

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Histiocytic and Dendritic Cell Disorders

Faramarz Naeim

Histiocytes/macrophages and most subtypes of dendritic cells are derived from the hematopoietic stem cells and play an important role in the regulation of immune functions. Histiocytes/macrophages are derived from monocytes and are involved in different aspects of host defense and tissue repair, such as phagocytosis, cytotoxic activities, regulation of inflammatory and immune responses, and wound healing. Dendritic cells (DC) are primarily involved in antigen processing and antigen presentation to the B- and T-lymphocytes [1, 2]. There are three major subclasses of dendritic cells: Langerhans cells (LC), interdigitating dendritic cells (IDC), and follicular dendritic cells (FDC). LC and IDC, similar to histiocytes/macrophages, are derived from hematopoietic stem cells, whereas FDC are derived from mesenchymal cells in the follicular structures of the lymph nodes [3–5]. The immunophenotypic features of histiocytes/macrophages and subclasses of DC are presented in Table 21.1.

MONOCYTIC AND HISTIOCYTIC DISORDERS

Disorders of monocytes and histiocytes/macrophages are divided into three major categories: (1) functional defects, (2) reactive responses, and (3) neoplastic proliferations. Functional defects are mostly hereditary, such as lysosomal storage diseases. Reactive disorders are non-neoplastic conditions associated with hypoplasia, hyperplasia, or hyperactivation of the monocytic/histiocytic system. Neoplastic disorders are the

result of clonal proliferation of monocytic/histiocytic cells (see Chapters 8–11). A summary of monocytic and histiocytic disorders is demonstrated in Table 21.2.

In this section, the following entities are discussed as examples of monocytic and histiocytic disorders:

Functional disorders

- Gaucher disease (GD)
- Niemann–Pick disease (NPD)
- Chediak–Higashi syndrome (CHS)

Reactive responses

- Monocytopenia and monocytosis
- Histiocytic proliferations
- Hemophagocytic histiocytosis

Neoplastic disorders

- Histiocytic sarcoma
- Dendritic cell tumors

Gaucher Disease

Gaucher disease (GD) is an inherited autosomal recessive inborn error of metabolism resulting in the accumulation of glucocerebroside (glucosylceramide) in macrophages. GD is the most common lysosomal storage disease occurring in about 1 in 75,000 births worldwide, mostly affecting the Ashkenazi Jews [6–9]. Lysosomal storage diseases are a group of disorders caused by deficiencies of lysosomal enzymes necessary for the degradation of glycolipids and glycoproteins (Table 21.3).

TABLE 21.1 Immunophenotypic features of macrophages/histiocytes and subclass of dendritic cells.

Markers	MP	LC	IDC	FDC
CD1a	—	+	—	—
CD4	+	+	—	—
CD21	±	—	—	+
CD35	±	—	—	+
CD45	+	+	±	—
CD68	+	±	±	—
CD207 (Langerin)	—	+	—	—
HLA-DR	+	+	+	—
S-100	—	+	+	—
FcR	+	+	—	—
Lysozyme	+	±	±	—
NSE	+	—	—	—

MP: macrophage/histiocyte, LC: Langerhans cells, IDC: interdigitating dendritic cells, FDC: follicular dendritic cell, FcR: Fc IgG receptors, NSE: non-specific esterase.

TABLE 21.2 Disorders of monocytes and histiocytes.

<i>Functional disorders</i>
1. Lysosomal disorders
2. Chronic granulomatous disease
3. Defective monocyte chemotaxis
4. Others
<i>Reactive disorders</i>
1. Monocytopenia
2. Monocytosis
3. Granulomatous disorders
4. Hemophagocytosis
5. Sinus histiocytosis with massive lymphadenopathy
6. Others
<i>Neoplastic disorders</i>
1. Chronic myelomonocytic leukemia
2. Acute myelomonocytic leukemia
3. Acute monocytic leukemia
4. Histiocytic sarcoma

Pathogenesis and Molecular Genetics

GD results from deficiency of the lysosomal enzyme glucocerebrosidase (or acid beta-glucosidase) [6, 10, 11]. The deficiency is secondary to mutations in the glucocerebrosidase gene located on chromosome 1q21 [12]. More than 180 distinct mutations are listed in the Human Mutation Database for glucocerebrosidase gene, which are mostly point mutations. Three major mutant alleles are

TABLE 21.3 Lysosomal storage disease.*

<i>Sphingolipidoses</i>
Gaucher disease
Niemann–Pick disease
Farber disease
Fabry disease
Krabbe disease
Metachromatic leukodystrophy
GM1 gangliosidosis
GM2 gangliosidosis (Tay–Sachs disease)
<i>Mucopolysaccharidoses</i>
Mucopolysaccharidosis I (Hurler disease)
Hunter disease
Sanfilippo disease
Morquio disease
Maroteaux–Lamy syndrome
Multiple sulfatase deficiency
<i>Glycoproteinoses</i>
Sialidosis
Fucosidosis
Mannosidosis
Aspartylglycosaminuria
<i>Mucolipidoses</i>
Mucopolipidosis II
Pseudo-Hurler polydystrophy
Mucopolipidosis IV
<i>Others</i>
Cystinosis
Pompe disease
Wolman disease

*Adapted from Ref. [20].

identified in affected patients: *N370S*, *L444P*, and *84GG* [6, 13–15]. These three mutations account for >90% of alleles in Ashkenazi patients.

The glucocerebrosidase deficiency leads to accumulation of glucocerebroside and other glycolipids in the macrophages leading to organomegaly, osteopenia, and cytopenia.

Pathology

Morphology

The morphologic features are characterized by the accumulation of glycolipid-laden macrophages, known as *Gaucher cells*, in the spleen, liver, bone marrow, and other tissues (Figures 21.1 and 21.2). Gaucher cells have abundant cytoplasm containing a large amount of hydrophobic glucocerebroside molecules in bilayered membranous sheets [16]. For this reason, the cytoplasm appears striated, like a wrinkled tissue paper. By electron microscopy, Gaucher cells reveal spindle- or rod-shaped, membrane-bound cytoplasmic inclusions consisting of numerous small, tubular structures measuring 13–75 nm in diameter [17].

The bone marrow biopsy sections demonstrate focal, interstitial, or diffuse accumulation of the Gaucher

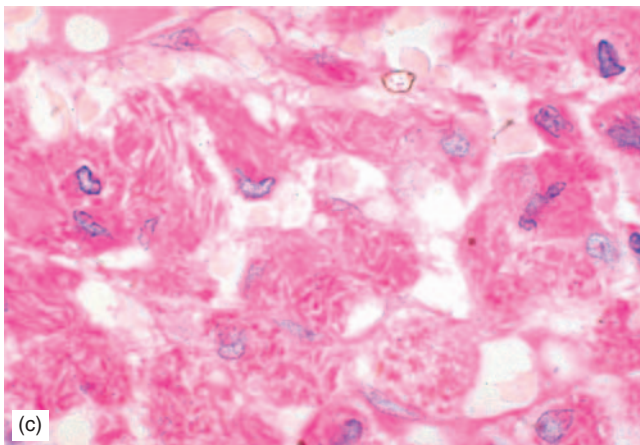
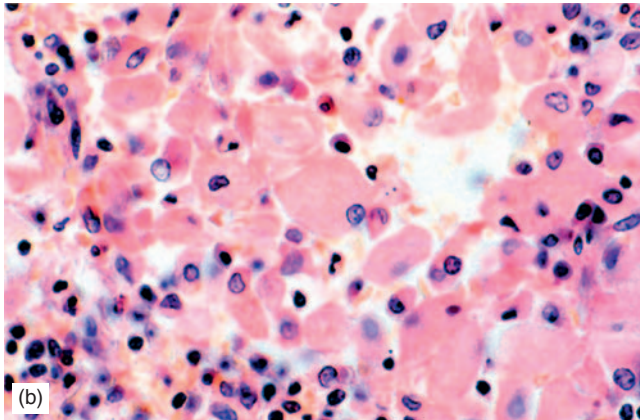
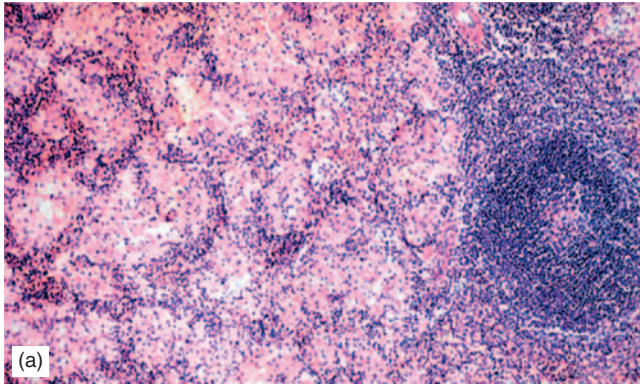


FIGURE 21.1 Gaucher disease. Section of spleen demonstrates diffuse infiltration of the red pulp by Gaucher cells: (a) low power and (b) high power. PAS stain shows accumulation of glucocerebroside molecules in membrane sheets creating cytoplasmic striation (c).

cells, sometimes associated with fibrosis (Figure 21.2). Accumulation of Gaucher cells is also present in the splenic red pulp in virtually all patients [18]. Gaucher cells are also found in the centrilobular and portal areas of the liver, sometimes in association with hepatic fibrosis [19].

Immunophenotype and Special Stains

The Gaucher cells are glucocerebroside-laden macrophages and, therefore, demonstrate expression of monocyte-associated

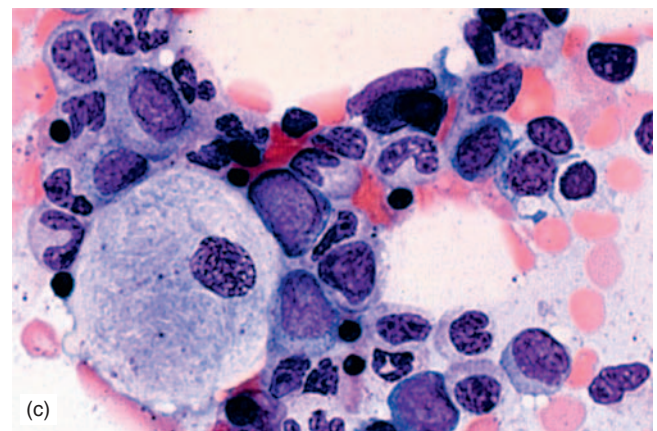
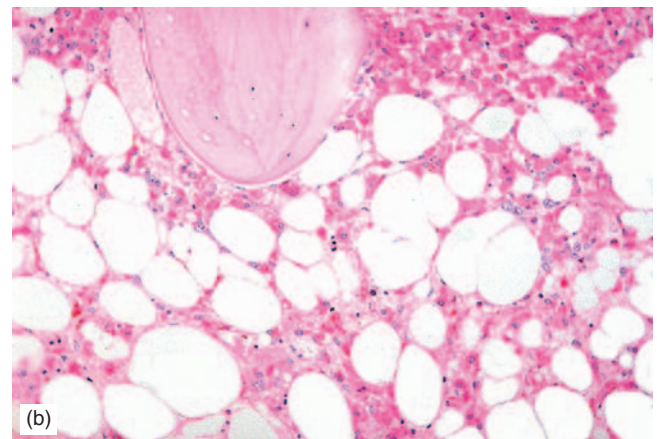
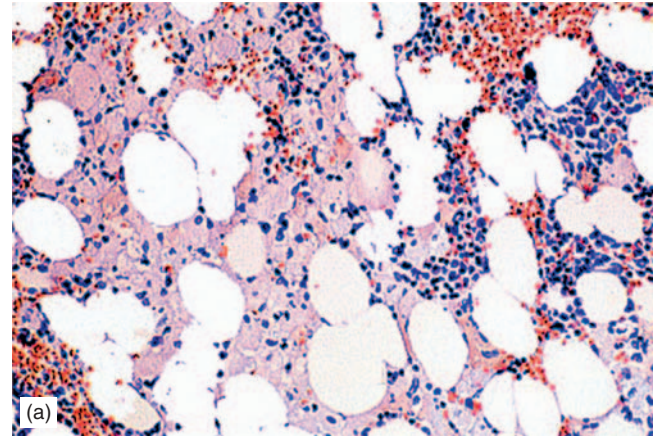


FIGURE 21.2 Gaucher disease. Bone marrow section demonstrates interstitial infiltration by Gaucher cells: (a) H&E, and (b) PAS stains. Bone marrow smear (c) shows one Gaucher cell characterized by abundant cytoplasm with striation.

markers such as CD45, lysozyme, and CD68 by immunohistochemical stains (Table 21.1). They are typically negative for CD1a and S-100 protein. The Gaucher cells are PAS-positive and demonstrate a strong acid phosphatase activity, which is usually resistant to tartaric acid inhibition. They are also Sudan Black B and non-specific esterase (NSE) positive [20].

Clinical Aspects

There are three types of GD. All three types share splenomegaly, hepatomegaly, bone marrow involvement, anemia, and skeletal changes. They differ in the presence or lack of neurologic manifestations, type of mutation, ethnic predilection, and clinical outcome (Table 21.4).

Type 1 GD represents about 90% of the patients with GD and is predominantly seen in the Ashkenazi Jewish population. The disease may occur at any age, but about 70% of the patients are diagnosed by the age of 20 years [21, 22]. Hepatosplenomegaly is common and there is no neurologic manifestation. Mild-to-moderate degree of anemia and thrombocytopenia is present, mainly due to hypersplenism. Liver enzymes, serum angiotensin converting enzyme, and acid phosphatase levels may be elevated. The most common mutation is *N370S*. The clinical course is variable with an overall more rapid progression in children than in adults [23].

Type 2 GD is the rarest form and is also known as acute neuronopathic GD or infantile cerebral GD. It usually occurs in the first year of life and is characterized by extensive visceral involvement and rapidly progressive neurologic deterioration, including oculomotor dysfunction and bulbar palsy [24]. The average survival is <12 months [25].

Type 3 GD is a subacute or chronic neuropathic form with a later onset and more variable course than type 2 GD. A subtype of this category (type 1a) which is associated with *L444P* mutation, progressive dementia, and ataxia has been described in Norrbottnian region of Sweden (Table 21.3) [26].

Diagnosis is confirmed by the demonstration of reduced glucocerebrosidase activity in peripheral leukocytes and/or the presence of mutation by molecular genetic studies [27, 28].

Enzyme replacement therapy with recombinant glucocerebrosidase (imiglucerase) is the treatment of choice for most symptomatic type 1 patients [29]. Substrate reduction therapy, such as treatment with miglustat, is used for those patients who cannot afford the high expense of enzyme replacement therapy. Miglustat is an inhibitor of glycosylceramide synthesis and reduces glycolipid accumulation [9]. Splenectomy and bone marrow transplantations are other alternative therapeutic approaches [30, 31].

Differential Diagnosis

The differential diagnosis includes those conditions that are associated with splenomegaly and cytopenia, such as leukemia, lymphoma, collagen vascular diseases, and other lysosomal storage diseases. The diagnosis of GD is usually made by the detection of Gaucher cells in bone marrow and is confirmed by the reduced leukocyte glucocerebrosidase activity and molecular genetic studies.

Niemann–Pick Disease

Niemann–Pick disease represents a group of autosomal recessive disorders that are associated with tissue accumulation of sphingomyelin, splenomegaly, and manifestation of variable degrees of neurologic defects [32, 33].

Pathogenesis and Molecular Genetics

This group of disorders has been associated with mutations in three different genes: (1) sphingomyelin phosphodiesterase 1

TABLE 21.4 Classification of Gaucher disease.

Features	Type 1	Type 2	Type 3
Onset	Variable	First year	Childhood
Anemia	+	+	+
Thrombocytopenia	+	—	—
Splenomegaly	+	+	+
Hepatomegaly	+	+	+
Bone marrow involvement	+	+	+
Skeletal changes	+	±	+
Neurologic manifestations	—	+	+
Mutations	<i>N370S</i>	Diverse	<i>L444P</i>
Ethnic predilection	Ashkenazi Jews	None	Norrbottnian, Sweden
Progression	Slow	Rapid	Variable

gene (*SMPD1*) mapped at chromosome 11p15, (2) Niemann–Pick C1 gene (*NPC1*) on chromosome 18q11–q12, and (3) Niemann–Pick C2 gene (*NPC2*) on chromosome 14q24.3 (Table 21.5). Mutations of *SMPD1* are most prevalent in the Ashkenazi Jews. Three *SMPD1* mutations, R496L, L302P, and fsP330, account for >90% of the type 1 NPD [34, 35]. These mutations lead to the deficiency of SMPD enzyme and accumulation of lysosphingomyelin in the macrophages and the neural tissues. Lysosphingomyelin is believed to be toxic to the nervous system [35].

The *NPC1* gene product is localized in vesicles that transiently interact with cholesterol-laden lysosomes to facilitate sterol and probably glycolipid relocation [36, 37]. The *NPC2* gene encodes a small soluble lysosomal cholesterol-binding protein [33, 38]. The *NPC1* and *NPC2* mutations, therefore, play a role in the accumulation of cholesterol and glycolipids in neurons and other cells [35].

Pathology

Morphology

The characteristic morphologic feature of NPD is the presence of foamy cells in various tissues secondary to the duplication and expansion of the sphingomyelin-laden lysosomal system. The expansion of lysosomal structure may eventually lead to the total occupation of the cytoplasmic space, creating a foamy appearance of the affected cells, known as “Niemann–Pick cells” (NP cells) (Figures 21.3 and 21.4). By electron microscopy, the NP cells are loaded with lysosomes, which may appear as membrane-bound wavy concentric structures or homogenous (washed out or lucent) deposits [20, 39]. NP cells consist of macrophages in the spleen, bone marrow and lymph nodes, endothelial cells, neurons, Schwann cells, and retinal cells [33].

TABLE 21.5 Major classes of Niemann–Pick disease.

NPD	Gene	Chromosome
Type 1A	Sphingomyelin phosphodiesterase 1 gene (<i>SMPD1</i>)	11p15
Type 1S	Sphingomyelin phosphodiesterase 1 gene (<i>SMPD1</i>)	11p15
Type 2S	Niemann–Pick C1 gene (<i>NPC1</i>) or Niemann–Pick C2 gene (<i>NPC2</i>)	18q11-q12 14q24.3

The bone marrow biopsy sections reveal focal, interstitial, or diffuse accumulation of the NP cells (Figures 21.3 and 21.4). The accumulation of NP cells is also present in the splenic red pulp in virtually all patients and may be demonstrated in the centrilobular and portal areas of the liver and lymph node sinuses [18, 20].

Immunophenotype and Special Stains

The NP cell accumulation in the hematopoietic tissues such as bone marrow, spleen, and lymph nodes are sphingomyelin-laden macrophages and, therefore, demonstrate expression of monocyte-associated markers such as CD45, lysozyme, and CD68 by immunohistochemical stains. They are typically negative for CD1a and S-100 protein (Table 21.1). The NP cells are Sudan Black B and NSE positive and contain lipopigment that stains intensively with iron hematoxylin [20, 40]. Cresyl violet and PAS stains are either negative or weakly positive [20, 40]. Filipin and BC theta stains are helpful in demonstrating unesterified lysosomal cholesterol droplets in the fibroblast cell culture or neurons by fluorescence or confocal microscopy [41, 42].

Clinical Aspects

Currently, NPD is divided into three major types: 1A, 1S, and 2S [33, 35].

Type 1A is the acute neuronopathic form (formerly known as type A) and the most common type of NPD with the highest incidence among Ashkenazi Jews. The affected children, usually >1 year of age, present with hepatosplenomegaly, feeding difficulties, and loss of motor skills with a progressive clinical downhill and death within 2–3 years [43]. Type 1A is caused by mutations of *SMPD1* gene (Table 21.6). Decreased serum HDL, increased serum LDL, and hypertriglyceridemia are frequent laboratory findings [43].

Type 1S is a chronic non-neuronopathic form of NPD which occurs during infancy or childhood (formerly called type B). It is characterized by hepatosplenomegaly, short stature, delayed skeletal maturation, and ocular abnormalities. The affected children usually survive into their adulthood [44]. In type 1S, the *SMPD1* enzyme activity is partially preserved, and decreased serum HDL, increased serum LDL, and hypertriglyceridemia may be present. Type 1C is a subtype of this category representing the adult non-neuronopathic form associated with hepatosplenomegaly.

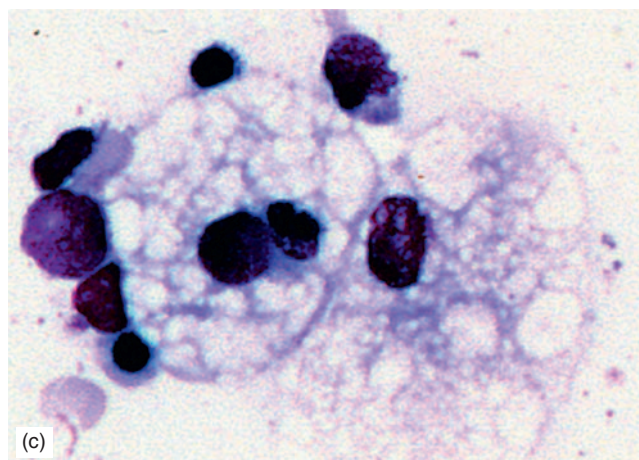
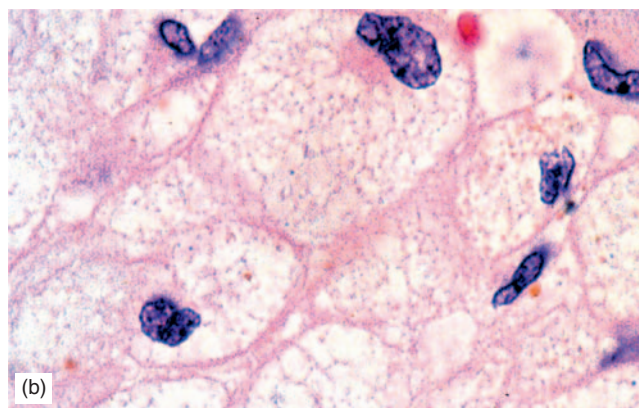
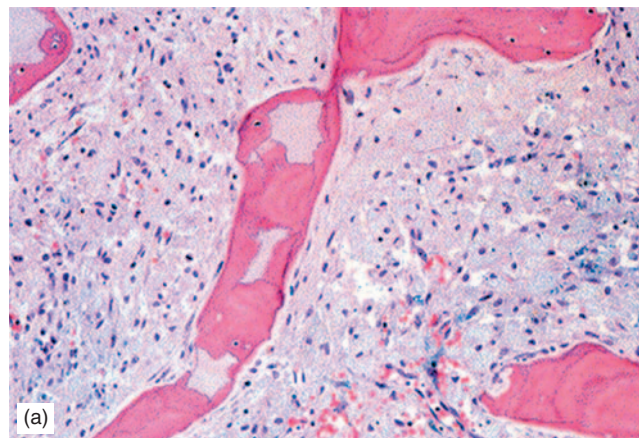


FIGURE 21.3 Niemann–Pick disease. Bone marrow biopsy section demonstrates diffuse infiltration of large vacuolated histiocytes: (a) low power and (b) high power views. Two large vacuolated histiocytes are shown in bone marrow smear (c).

Type 2S represents the abnormalities of intracellular transport of cholesterol and its sequestration in lysosomes [35]. This functional defect is the result of mutations in *NPC1* and *NPC2* genes (Table 21.6). Most patients demonstrate neurologic disease with a late infantile or juvenile onset. Cerebellar involvement, dystonia, ophthalmoplegia, and seizures are among neurologic manifestations [33, 35]. Type 2S consists of two subtypes: type C (more frequent, younger age

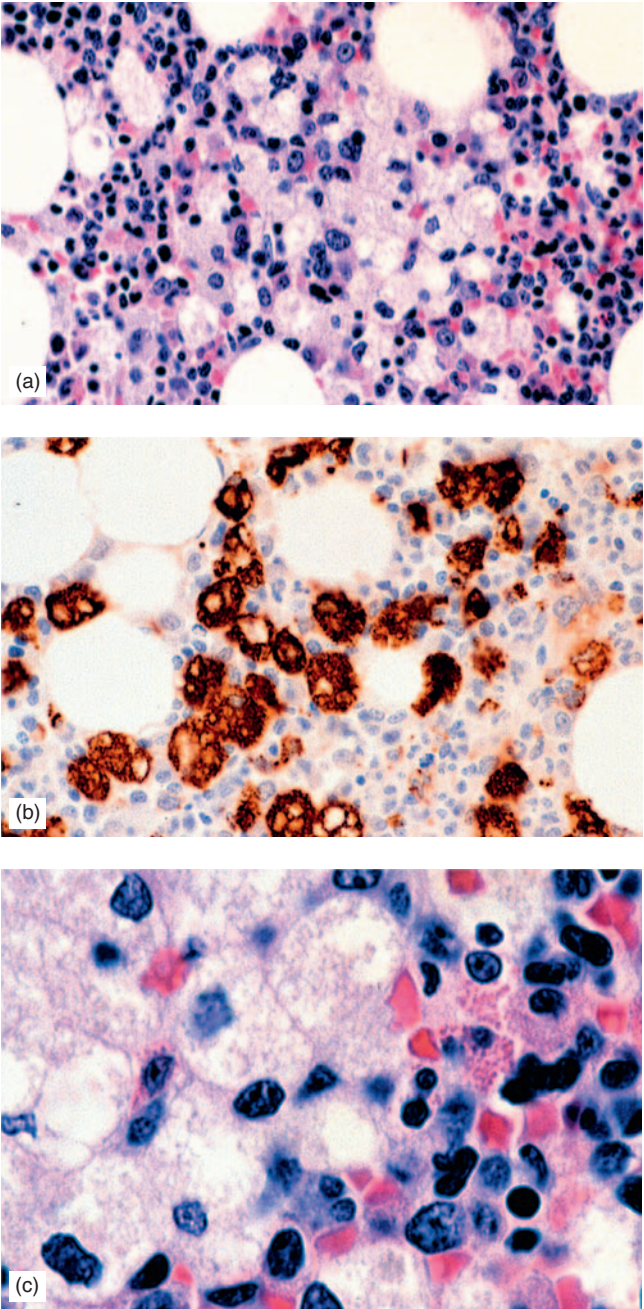


FIGURE 21.4 Niemann–Pick disease. Bone marrow biopsy section demonstrates clusters of finely vacuolated histiocytes (a) that are CD68-positive (b). A high power view is shown in (c).

of onset, and more aggressive) and type D (later age of onset and slower progress).

There is no effective treatment for NPD. Cholesterol-lowering drugs reduce the hepatic-free cholesterol levels but do not change the clinical course. Bone marrow transplantation does not reverse the neurologic symptoms [45, 46].

Differential Diagnosis

The differential diagnosis includes neurologic disorders and conditions that are associated with hepatosplenomegaly.

TABLE 21.6 Conditions associated with monocytosis.*

<i>Infections</i> <ol style="list-style-type: none">1. Tuberculosis2. Syphilis3. Subacute bacterial endocarditis4. Cytomegalovirus infection5. Disseminated candidiasis6. Others
<i>Inflammatory and immune-associated disorders</i> <ol style="list-style-type: none">1. Myositis2. Temporal arteritis3. Polyarteritis nodosa4. Rheumatoid arthritis5. Inflammatory bowel diseases6. Alcoholic liver disease7. Others
<i>Hematologic disorders</i> <ol style="list-style-type: none">1. Neoplastic disorders<ol style="list-style-type: none">(a) Chronic myelomonocytic leukemia(b) Acute myelomonocytic and acute monocytic leukemias(c) Lymphoma(d) Plasma cell myeloma2. Non-neoplastic conditions<ol style="list-style-type: none">(a) Hemolytic anemia(b) Chronic neutropenia(c) Postsplenectomy(d) Idiopathic thrombocytopenic purpura(e) Others
<i>Non-hematopoietic malignancies</i> <p>Drug-induced</p> <ol style="list-style-type: none">1. Chlorpromazine2. Ampicillin3. Glucocorticoids4. Others

*Adapted from Ref. [20].

Diagnosis is established by morphologic findings in biopsy sections, use of special stains (such as Filipin and BC theta stains), and molecular genetic studies demonstrating mutations of *SMPD1*, *NPC1*, or *NPC2*.

Chediak–Higashi Syndrome

Chediak–Higashi syndrome (CHS) is a rare autosomal recessive disorder characterized by severe immune deficiency, partial albinism, bleeding tendencies, and recurrent bacterial infections [47, 48]. The defective gene, *LYST*, is a lysosome-traffic regulator mapped at 1q42 [49, 50]. *LYST* is a large, highly conserved gene encoding a protein with 3,801 amino acids and a predicted molecular weight of 429kDa [51]. Multiple different mutations in CHS patients have been described, but molecular genetic testing is not yet readily available. The *LYST* gene is involved with intracellular protein trafficking, and its mutation may lead to impairment in the fusion of cytoplasmic vesicles [47, 48].

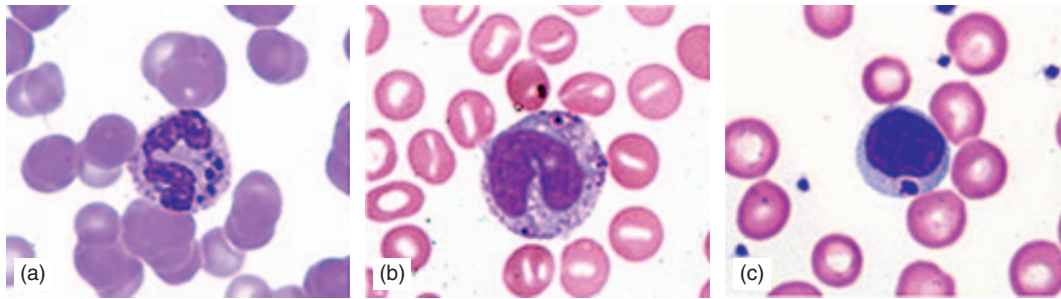


FIGURE 21.5 Chediak-Higashi syndrome. Cytoplasmic granules of variable sizes are present in neutrophils (a), monocytes (b) and lymphocytes (c).

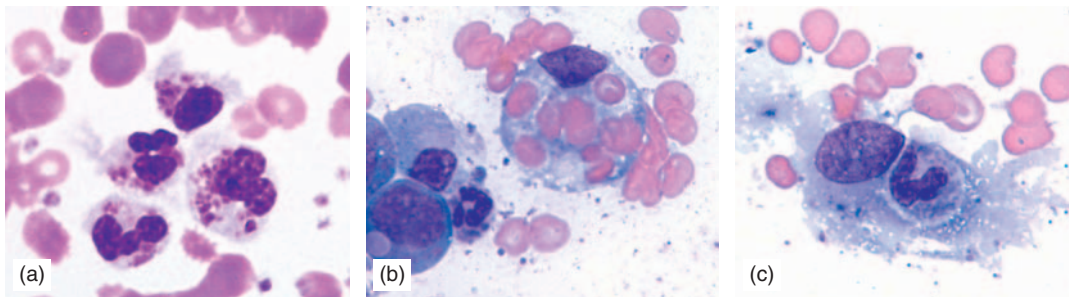


FIGURE 21.6 Chediak-Higashi syndrome in accelerated phase. Blood smear demonstrates several white cells with cytoplasmic granules (a). Bone marrow smear shows an erythrophagocytic histiocytes (b) and a histiocyte containing a neutrophilic band (c).

The morphologic hallmark of CHS is the presence of giant cytoplasmic granules in various cells. These granules include lysosomes (such as monocytes, granulocytes, cytotoxic T-, and NK-cells), melanosomes (melanocytes), and cytoplasmic granules in Schwann cells (Figures 21.5 and 21.6) [47, 52]. The platelets contain abnormal dense bodies [47].

Clinical features are presented in early childhood and reflect the functional defects in leukocytes, melanocytes, platelets, and Schwann cells and consist of recurrent pyogenic infection, partial oculocutaneous hypopigmentation, coagulation defect with petechiae, bruising and mucosal bleeding, and neurologic disturbances such as peripheral neuropathy and dysfunction of the spinal tract and the cerebellum [53]. The evolution to an “accelerated phase” had been reported in some cases characterized by T-cell lymphocytosis and hemophagocytic histiocytosis (Figure 21.6) [47, 52]. An association between EBV infection and accelerated phase has been observed, suggesting that the hemophagocytic lymphohistiocytosis (HLH) is EBV-induced [54, 55]. The treatment of choice is bone marrow transplantation, which improves leukocyte and platelet defects and immunologic problems [47].

Monocytopenia and Monocytosis

Monocytopenia is less frequent than monocytosis and occurs in aplastic anemia, hairy cell leukemia, severe thermal injuries, and treatment with corticosteroids [20, 56–58]. Decreased monocyte blood count has also been reported

in patients with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and AIDS [59, 60]. Cyclic neutropenia may be associated with intermittent monocytopenia [20].

Monocytosis is observed in a wide variety of conditions such as chronic and subacute infections, collagen vascular disorders, hematologic and non-hematologic malignancies, hemolytic anemia, and idiopathic thrombocytopenic purpura (Table 21.6). Certain drugs such as chlorpromazine, ampicillin, and tetrachloroethane may also induce monocytosis.

Reactive Histiocytic Proliferations

Reactive histiocytic proliferation is often associated with other inflammatory cells such as lymphocytes, plasma cells, eosinophils, and neutrophils. The reactive histiocytes may be diffusely intermixed with other inflammatory cells or may appear as aggregates of cohesive (epithelioid) histiocytes standing alone or accompanied by other inflammatory cells, making granulomas (see Chapter 5). Reactive histiocytic proliferations may be secondary to infectious diseases, autoimmune disorders, or malignancies, or may be idiopathic.

Infections

A wide variety of infections cause histiocytic proliferation predominantly in epithelioid clusters or granulomatous formation (see Chapter 5). For example, mycobacterial infections, syphilis, leprosy, Q fever, cat-scratch fever, and fungal

infections typically cause granulomatous formation in the bone marrow, lymph nodes, and other tissues (see Figures 5.5 and 5.6). Small clusters of epithelioid histiocytes are present in the lymph nodes infected with *Toxoplasma gondii* and occasionally may contain microorganism. Protozoan-laden epithelioid histiocytes are identified in the bone marrow, lymph nodes, and other tissues of patients with leishmaniasis (see Figure 5.8).

Autoimmune Disorders

Autoimmune disorders such as SLE and RA are sometimes associated with lymphadenopathy and increased histiocytic proliferations. In SLE, affected lymph nodes show follicular hyperplasia and areas of necrosis in paracortical areas (see Chapter 6). Necrotic areas are surrounded by histiocytes and other inflammatory cells [61, 62]. The enlarged lymph nodes in RA show follicular hyperplasia, sinus histiocytosis, interfollicular plasmacytosis, and deposition of PAS-positive, Congo-red-negative hyaline material (see Figure 6.1) [61, 63, 64].

Tumor-Associated

Histiocytes are one of the prominent background inflammatory cells in Hodgkin lymphoma. They may also appear as discrete epithelioid aggregates or granulomas. Histiocytic proliferation is also frequently observed in T-cell malignancies, sometimes associated with hemophagocytosis (discussed later). In the peripheral T-cell lymphoma of Lennert type, neoplastic T-cells are mixed with sheets or aggregates of epithelioid histiocytes (see Chapter 17). Metastatic carcinomas to lymph nodes may be associated with sinus histiocytosis.

Idiopathic

Sarcoidosis, Kikuchi disease, Erdheim–Chester disease, and sinus histiocytosis with massive lymphadenopathy are examples of reactive histiocytic proliferations with no known etiology (see Chapter 6).

Sarcoidosis is a rare granulomatous disorder, more prevalent in Blacks than in Caucasians and in women than in men [61]. Mediastinal and pulmonary hilar lymph nodes are most frequently affected, but other tissues and organs such as the bone marrow, liver, spleen, and lungs may also be affected. The affected tissues show multiple well-defined granulomas consisting of epithelioid histiocytes and multinucleated giant cells without significant necrosis (see Figure 6.9) [65].

Kikuchi disease is a rare histiocytic necrotizing lymphadenitis, more frequently seen in Asia than in Western countries [61]. It is more prevalent in young adult women. The affected lymph nodes show discrete or confluent eosinophilic areas consisting of phagocytic and non-phagocytic histiocytes, lymphocytes, immunoblasts, eosinophils, and plasma cells with rare or absent neutrophils (see Figure 6.14).

Erdheim–Chester disease is a rare condition characterized by a symmetrical sclerosis of lower extremities and involvement of many organs and tissues, including the lung, kidney, orbit, skin, pericardium, and retroperitoneum [66, 67]. The affected tissues show aggregates or sheets

of lipid-laden histiocytes and scattered multinucleated Touton giant cells (lipid-laden histiocytes in which multiple nuclei are grouped around a small island of cytoplasm) (see Figure 5.9).

Sinus histiocytosis with massive lymphadenopathy (Rosai–Dorfman disease) is a rare condition affecting children and young adults. It is characterized by massive, bilateral, painless, cervical lymphadenopathies, sometimes with extranodal involvement such as the skin, bone, upper respiratory tract, or central nervous system [61]. The cortical and medullary sinuses of the affected lymph nodes are markedly dilated and filled with large histiocytes with abundant, pale, vacuolated cytoplasm. These histiocytes show evidence of emperipolesis by containing intact lymphocytes, and less frequently, plasma cells, neutrophils, or erythrocytes (see Figure 6.7) [68].

Iatrogenic

Lymphangiogram-associated lipogranuloma (Figure 21.7), prosthetic- or implanted-induced foreign body reactions are among the examples of iatrogenic histiocytic proliferations [61, 69, 70].

Hemophagocytic Lymphohistiocytosis

Hemophagocytic lymphohistiocytosis (HLH) is characterized by proliferation of reactive non-dendritic histiocytes with evidence of hemophagocytic activities [71–74]. The hemophagocytosis is either associated with an underlying genetic disorder or secondary to an underlying infection, autoimmune or neoplastic process [75].

Etiology and Pathogenesis

The primary underlying pathophysiologic mechanism in HLH appears to be related to cytokine abnormalities, leading to uncontrolled accumulation of activated T-lymphocytes and histiocytes [76, 77]. High plasma levels of interferon-gamma (IFN- γ), tumor necrosis factor-alpha (TNF- α), interleukin (IL)-1, IL-2, IL-6, IL-10, IL-12, and IL-18 have been reported in patients with HLH [77–79]. Mutation of perforin gene (*PRF1*) plays an important role in the pathogenesis of HLH in a significant proportion of patients. Perforin protein is stored in the cytoplasmic granules of large granular lymphocytes, monocytes, and other hematopoietic precursors, and plays an important role in the formation of pores in the membrane of target cells. Once the target cell membrane is perforated, granzymes and other cytolytic components are able to enter the target cells. A defective perforin protein results in the failure of killing the target cells and removal of the antigenic stimulation by viruses or other infectious agents. The persistent stimulation of T-cells results in the production of large amounts of cytokines and activation of macrophages.

There are four major subtypes of hemophagocytic lymphohistiocytosis: (1) familial HLH, (2) infection-associated HLH, (3) hemophagocytosis associated with autoimmune and immunodeficiency disorders, and (4) cancer-associated HLH.

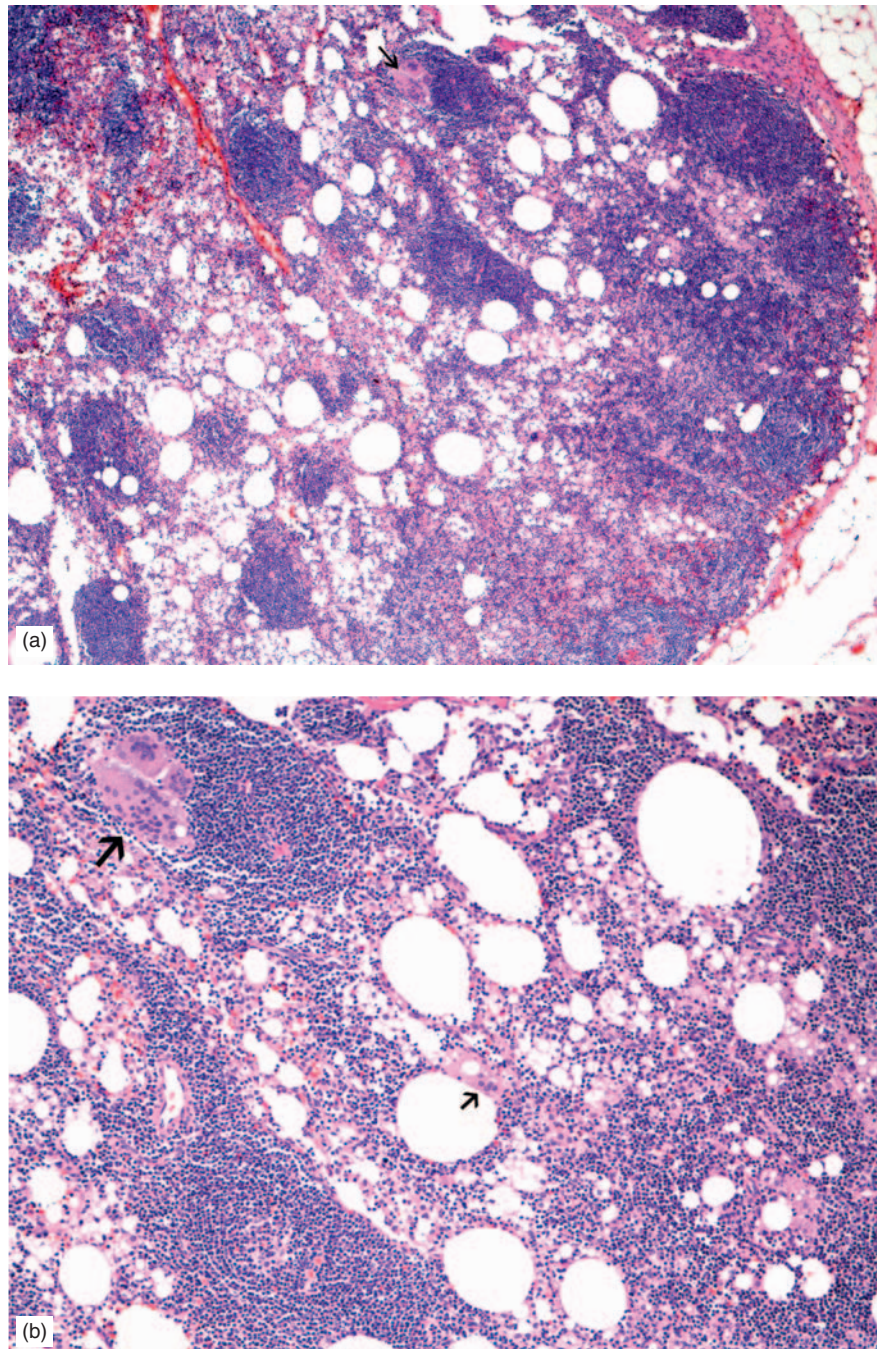


FIGURE 21.7 Lymphangiogram-associated lipogranuloma. Lymph node section reveals sinus histiocytosis with numerous foamy, lipid-containing histiocytes and the presence of multinucleated giant cells (arrows): (a) low power, (b) intermediate power, and (c) high power views. Courtesy of G. Pezeshkpour, M.D., Department of Pathology, VA Greater Los Angeles Healthcare System.

Familial HLH is an autosomal recessive disorder affecting infants from birth to 18 months of age [80, 81]. Mutation of *PRF1*, *UNC13D*, and *STX11* genes is most frequently reported in the familial type [82–84].

Infection-associated HLH has been reported in a wide variety of viral and bacterial infections including EBV, CMV, parvovirus, herpes simplex, varicella zoster, measles, HIV, tuberculosis, gram-negative bacteria, as well as fungal and parasitic infections [72, 75, 85–88].

Hemophagocytosis associated with autoimmune and immunodeficiency disorders have been observed in SLE, RA, polyarteritis nodosa, pulmonary sarcoidosis, Sjogren's syndrome, and a wide variety of immunodeficiency conditions such as X-linked lymphoproliferative syndrome, Kawasaki disease, and Chediak–Higashi syndrome [75, 89–92].

Cancer-associated HLH has been reported primarily in T-cell lymphoid malignancies as well as NK-cell leukemia and B-cell lymphoma [93–96].

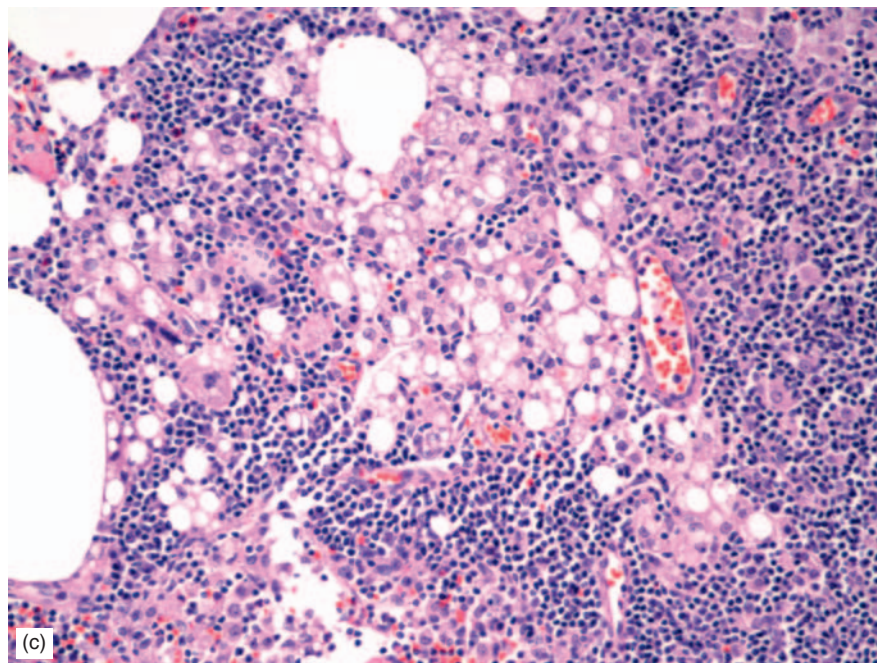


FIGURE 21.7 (Continued)

Pathology

Morphology and Laboratory Findings

HLH is usually a systemic disorder involving various organs with predilection of bone marrow, spleen, liver, and lymph nodes (Figures 21.8–21.10). Other sites of involvement include meninges, lungs, gastrointestinal tract, thymus gland, and genitourinary system. Cutaneous manifestations are rare [97]. The involved tissues show increased number of a mixture of lymphocytes and histiocytes in focal, interstitial, sinusoidal, or diffuse patterns. The histiocytes show abundant finely granular or vacuolated cytoplasm. The nucleus is bland, round, oval, or cleaved, and the nucleoli are inconspicuous. Numerous histiocytes show hemophagocytosis, which is predominantly erythrophagocytosis, and also includes phagocytosis of other hematopoietic cells such as platelets, neutrophils, and lymphocytes. There is a lack of significant cytologic atypia, and mitotic figures are absent or rare. Bone marrow appears to be the best tissue resource for the establishment of the diagnosis. However, in some cases, repeated bone marrow samples are required to document hemophagocytosis.

Other findings include cytopenia, hypertriglyceridemia and/or hypofibrinogenemia, low or absent NK-cell activity, and elevated serum ferritin concentration and soluble CD25 (Table 21.7) [98].

Immunophenotype and Cytochemical Stains

Histiocytes are of the non-dendritic cell type and therefore express CD68 and lysozyme. They are negative for CD1a and S-100 by immunohistochemical stains (Table 21.1). Histiocytes show positive reactions for NSE, acid phosphatase, and alpha-1-antitrypsin.

Molecular and Cytogenetic Studies

Mutation of *PRF1*, *UNC13D*, and *STX11* genes is most frequently reported in the familial type [82, 83]. In one study, 30% of the German patients and 80% of patients from Turkish origin with familial HLH showed *PRF1*, *UNC13D*, or *STX11* mutation [82]. The *PRF1* gene is mapped to the long arm of chromosome 10 in the 10q21-22 region [77].

Clinical Aspects

The initial signs and symptoms of HLH may simulate systemic infection, hepatitis, or encephalitis [75]. The most common clinical signs include fever and hepatomegaly (~90%), splenomegaly (~80%), neurologic symptoms (~45%), and lymphadenopathy (~40%) [75]. The clinical outcome is poor, and delay in therapy may lead to irreversible multiorgan failure and death. The recommended treatment is based on the guidelines provided by HLH-94 protocol [99], which includes induction therapy by dexamethasone and etoposide, followed by cyclosporine and dexamethasone. With this protocol, the reported overall 3-year survival is about 55%. Hematopoietic stem cell transplantation is the treatment of choice [99].

Differential Diagnosis

HLH clinically may mimic multiple organ failure syndrome including respiratory, cardiovascular, hepatic, and renal failures. The CNS involvement may simulate encephalitis, and pancytopenia may suggest bone marrow failure or leukemia. The majority of the HLH cases are associated with underlying causes such as infection and autoimmune disorder of lymphoid malignancies. HLH is distinguished from LCH by the presence of numerous hemophagocytic cells, expression

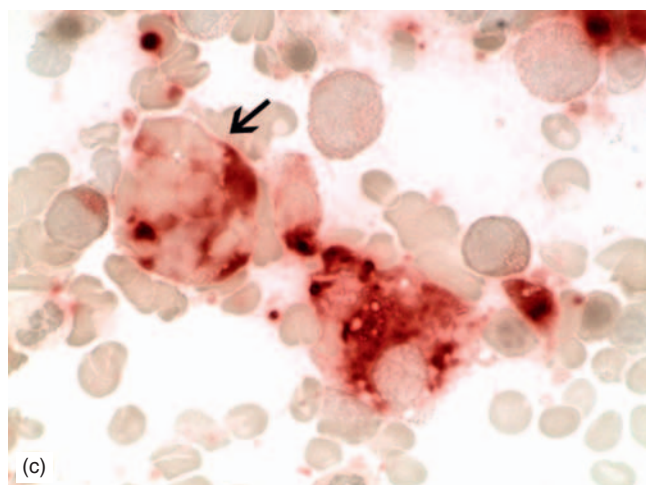
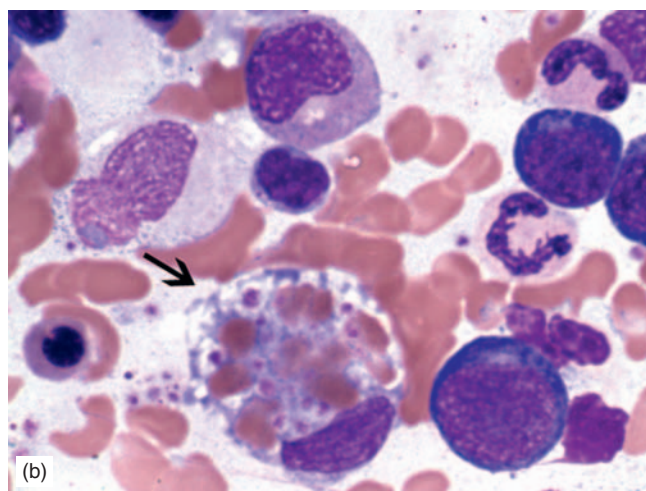
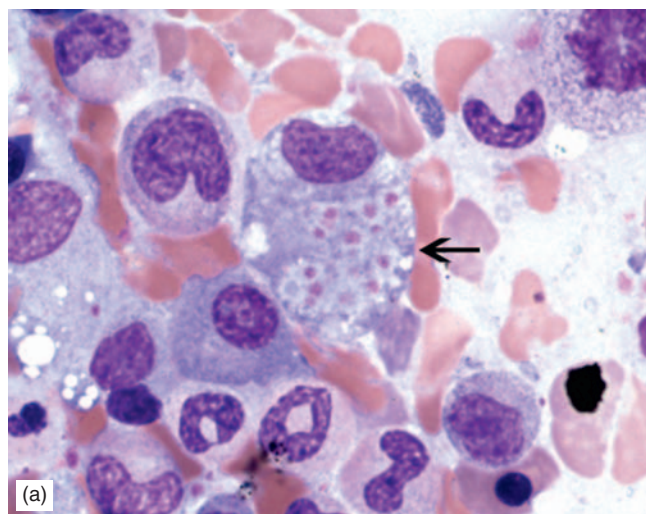


FIGURE 21.8 Hemophagocytosis. Bone marrow smear demonstrates histiocytes containing platelets (a, arrow) and erythrocytes (b, arrow). Immunohistochemical stain for CD68 shows a positive hemophagocytic histiocyte (c, arrow).

of NSE, lysozyme, and CD68, and lack of expression of CD1a and S-100. No Birbeck granules are demonstrated by electron microscopy (discussed later). Clinical manifestations and the presence of numerous hemophagocytic histiocytes separate HLH from other histiocytic disorders such as lysosomal storage diseases.

Histiocytic Sarcoma

Histiocytic sarcoma is a rare extramedullary malignant neoplasm demonstrating morphologic and immunophenotypic features of mature tissue histiocytes with lack of expression of lymphoid and dendritic-cell-associated markers. Acute myelogenous leukemias with monocytic differentiation are excluded [5, 100–102].

The etiology and pathogenesis are not known. The extranodal involvement is frequent. The infiltrating tumor cells show a diffuse growth pattern composed of large cells with abundant eosinophilic cytoplasm, round or irregular nuclei with vesicular chromatin, and one or more distinct nucleoli. Some cases may show significant pleomorphism with focal areas of spindle cells or the presence of multinucleated giant cells, and/or evidence of hemophagocytosis [100]. Histiocytic sarcoma may resemble diffuse large B-cell lymphoma, anaplastic large cell lymphoma, carcinoma, or melanoma [100–102].

Immunohistochemical stains show strong positivity for CD68 and CD163. Lysozyme stain may be weakly positive and there may be focal positivity for S-100 protein. The flow cytometric studies reveal a group of cells expressing CD45, CD4, CD11c, CD14, CD64, and HLA-DR. CD33 and CD34 are negative [5, 100, 102].

Histiocytic sarcoma is an aggressive disease with a poor response to chemotherapy.

DENDRITIC CELL DISORDERS

Langerhans Cell Histiocytosis

Langerhans cell histiocytosis (LCH), previously referred to as histiocytosis X, is a neoplasm of Langerhans dendritic cells characterized by the expression of CD1a and S-100 and the presence of ultrastructural cytoplasmic Birbeck granules [5]. LCH represents a wide variety of clinical manifestations with their specific terminology such as *eosinophilic granuloma* (solitary bone or extraosseous lesions), *HandSchuller-Christian* (multifocal, unisystem) disease, *Letterer-Siwe* (multiple organ system) disease, and *Hashimoto-Pritzker* (spontaneously resolving) syndrome (Table 21.8) [5, 102–108].

Etiology and Pathogenesis

The etiology and pathogenesis of LCH are not known. The LC proliferation appears to be clonal, but it is still not clear whether this clonal proliferation is induced by environmental conditions (viruses, cytokines) or genetic predispositions,

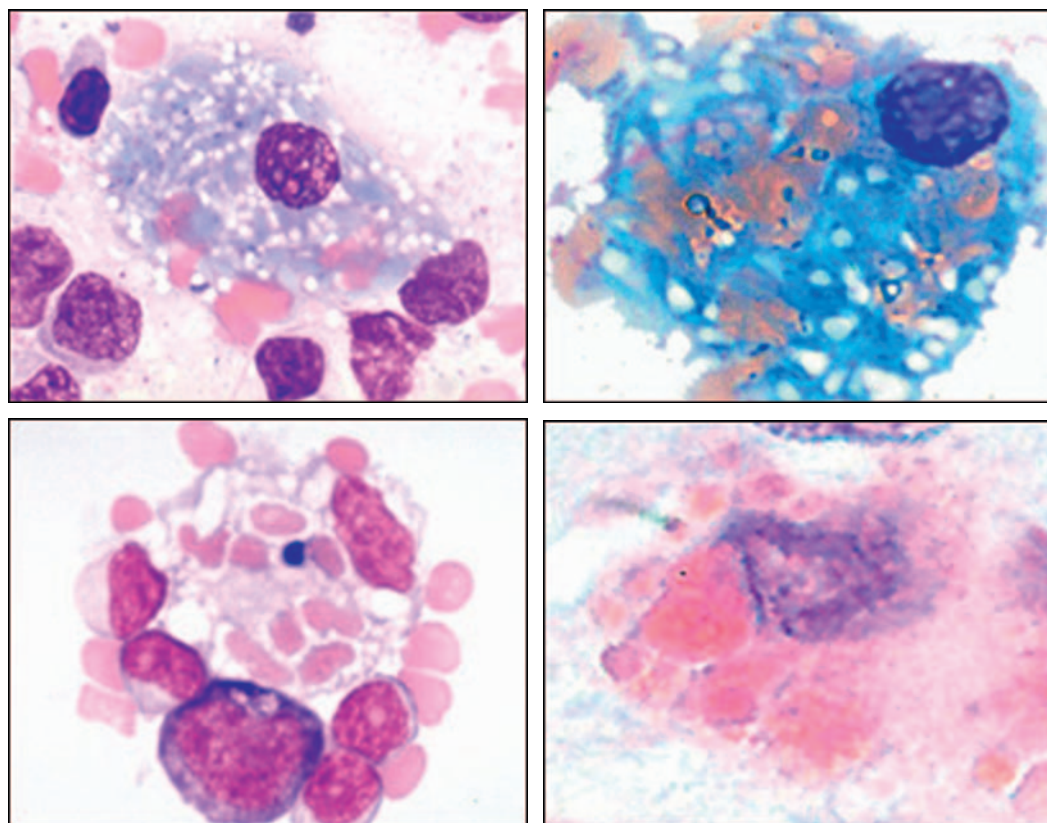


FIGURE 21.9 Erythrophagocytic histiocytes in bone marrow.

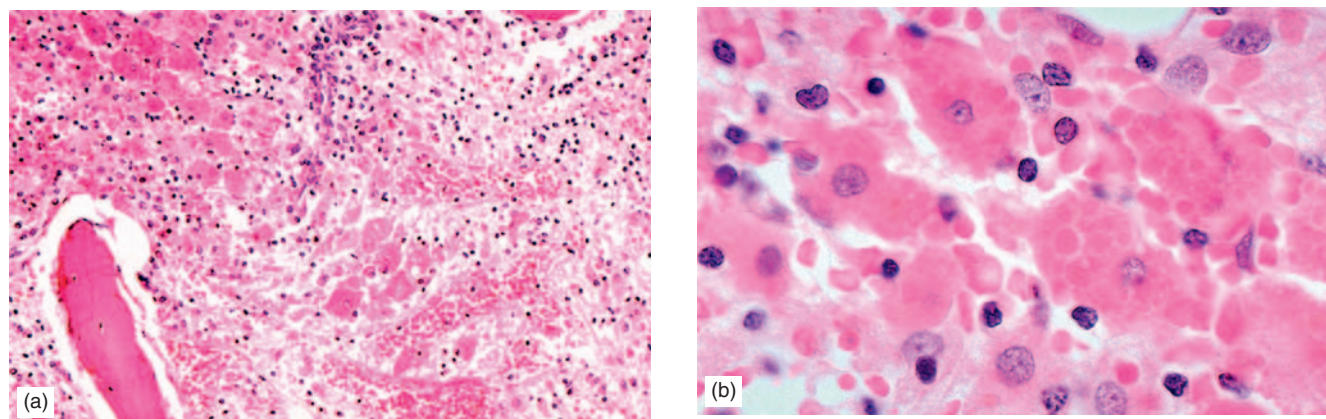


FIGURE 21.10 Bone marrow sections after chemotherapy and/or irradiation may show erythrophagocytic histiocytes: (a) low power and (b) high power views.

or both [108–113]. The significance of human herpes virus-6 (HHV-6) or EBV infections in the pathogenesis of LCH is still not clear [114–116].

Some investigators believe that development of LCH is primarily the result of an immunologic dysfunction and elevated levels of numerous cytokines such as GM-CSF, IFN- γ , IL-1, IL-4, and IL-10 [117]. It has been suggested that aberrant expression of certain chemokine receptors such as CCR6, CCR7, and CCL20/MIP-3 α may play a role in pathogenesis of LCH [109, 118].

An association between HLA and LCH has been reported. For example, HLA-DRB1 was frequently found

in the Nordic patients with unisystem LCH, whereas HLA-Cw7 and HLA-DR4 were found more prevalent in Caucasian LCH patients with solitary bone lesions [119, 120]. Several molecular cytogenetic abnormalities have been reported in patients with LCH, such as gains of DNA copy number on chromosomes 2q, 4q, and 12 and losses of DNA sequences on chromosomes 1p, 5, 6, 16, and 22q [121].

The cells of LCH are immature dendritic cells with reduced or absent antigen presenting capability and loss of ability to migrate from the involved tissue [122]. Altered cellular biology in LCH is also evident by the overexpression of Bcl-2, Ki-67, TGF- β R1, p53, RB, p16, and p21 [123, 124].

Pathology

Morphology

In the H&E sections, LC appear as large cells with abundant eosinophilic or pale cytoplasm, grooved, folded, indented, or convoluted nuclei with fine chromatin, and inconspicuous nucleoli (Figures 21.11 and 21.12). Mitotic figures are rare and hemophagocytosis is infrequent. The involved tissues are focally or diffusely infiltrated by the LC, often with increased eosinophils. Multinucleated giant cells and areas of necrosis may be present (Figure 21.12a). In more chronic lesions, cellular elements are replaced by fibrosis. Bone marrow smears or tissue touch preparations may show the presence of large histiocytic cells with abundant gray-blue vacuolated or finely granular cytoplasm (Wright’s stain), sometimes with dendritic cytoplasmic projections (Figure 21.12c).

TABLE 21.7 Diagnostic criteria for hemophagocytic lymphohistiocytosis.*

Major criteria
1. High fever for ≥7days
2. Splenomegaly
3. Cytopenia
4. Hypertriglyceridemia or hypofibrinogenemia
5. Hemophagocytosis
Minor criteria
A. Low or absent NK-cell activity
B. Serum ferritin level >500 µg/L
C. Soluble CD25 >2400 U/mL
Diagnosis of HLH requires all 5 major criteria or 4 major criteria plus (A), or 4 major criteria plus (B) and (C).

*Adapted from Ref. [98].

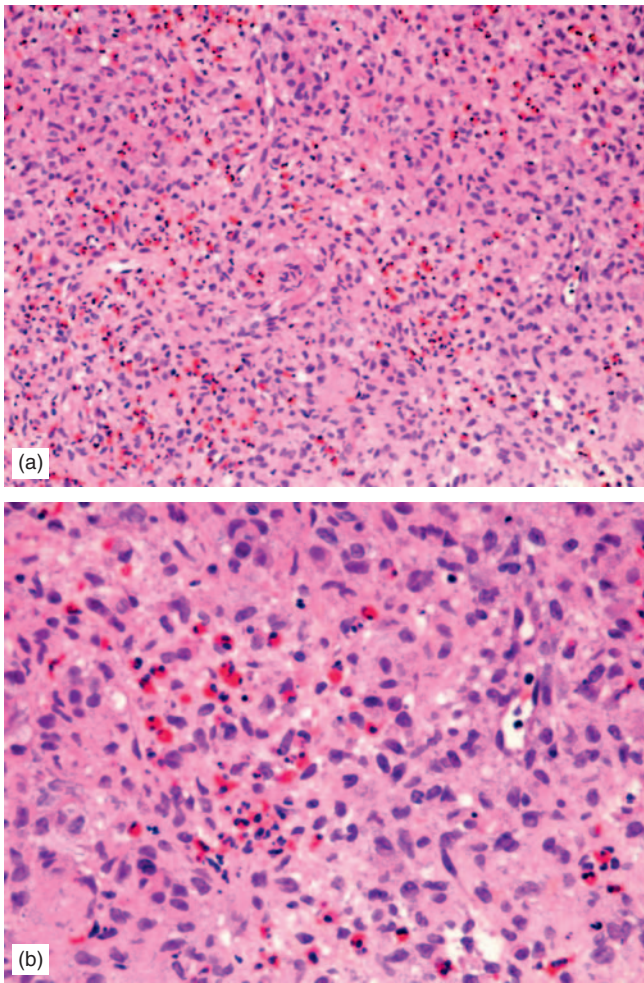


FIGURE 21.11 Langerhans cell histiocytosis. Bone marrow biopsy section demonstrates diffuse infiltration of large mononuclear cells with abundant eosinophilic cytoplasm. Scattered eosinophils are present: (a) low power and (b) high power views.

TABLE 21.8 Clinical classification of Langerhans cell histiocytosis.*

Type	Clinical features
Unifocal	Single site of involvement, most commonly bone Older children and adults Good prognosis
Multifocal single system	Multiple sites of involvement, most commonly bone Young children Intermediate prognosis
Multifocal multisystem	Multiple involved sites in more than one organ system Most commonly bone, skin, liver, spleen, and lymph nodes Children <2 years of age Poor prognosis
Congenital self-healing	Multiple skin lesions involving neonates and infants Self-healing involution
Pulmonary LCH	Young adult smokers Indolent, progression to pulmonary fibrosis

*Proposed by the Histiocyte Society (Favara B, *Med Pediatr Oncol* 1997) and adapted from Ref. [122].

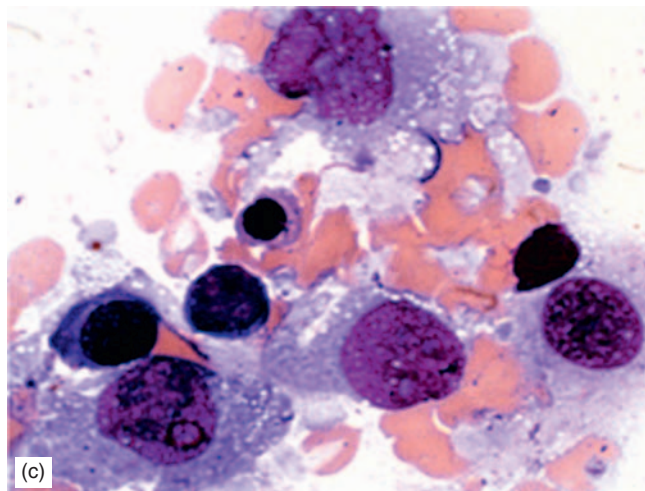
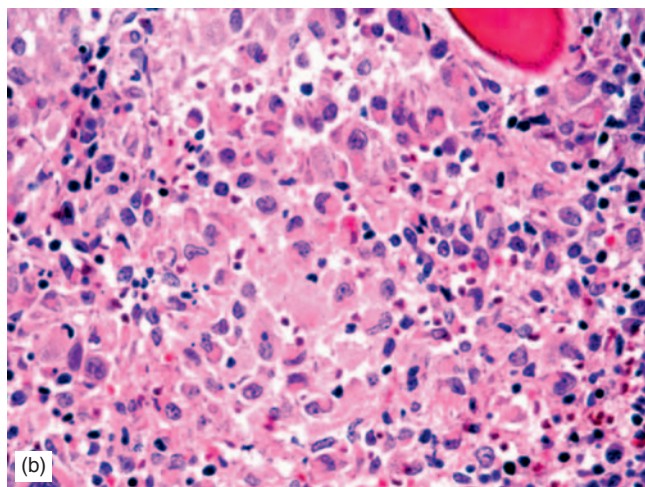
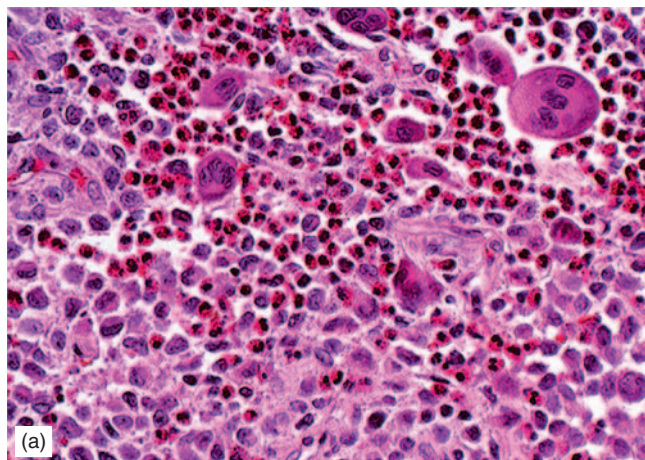


FIGURE 21.12 Langerhans cell histiocytosis. Bone marrow biopsy section (a and b) demonstrates diffuse infiltration of large mononuclear cells with abundant eosinophilic cytoplasm. Numerous eosinophils and scattered multinucleated giant cells are present (a). Bone marrow smear (c) shows several large cells with abundant finely vacuolated cytoplasm.

Electron microscopy demonstrates the characteristic cytoplasmic Birbeck granules. These granules are rod-shaped, often with the expanded end, resembling a tennis racket (Figure 21.13) [20, 125]. These structures consist of

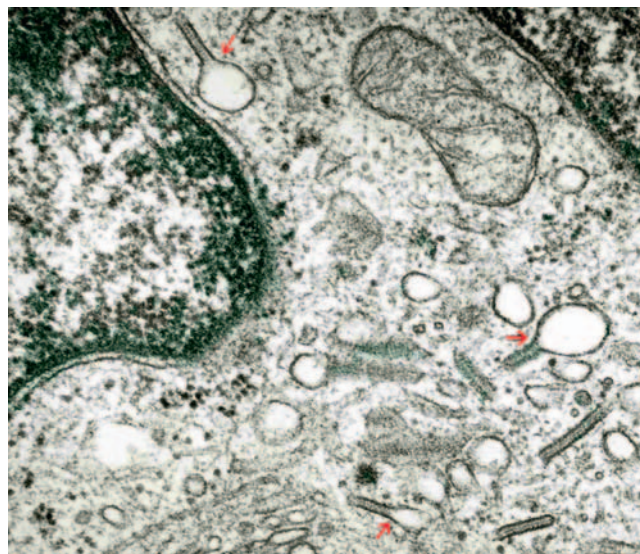


FIGURE 21.13 Electron micrograph of a Langerhans cell with Birbeck granules appearing like tennis rackets (arrows). Courtesy of Sunita Bhuta, M.D., Department of Pathology and Laboratory Medicine, UCLA Medical Center.

superimposed and zippered membranes. A protein known as “Langerin” (CD207) (see Table 3.1) is constitutively associated with Birbeck granules [125].

The most common single sites of involvement include the bone, skin, or lymph nodes. Multisystem disease presentation may include the liver, spleen, bone marrow, lung, and the endocrine, gastrointestinal, and central nervous systems [123].

Immunohistochemistry

The major immunophenotypic characteristics of LCH are similar to the normal LC and are demonstrated in Table 21.1. The expression of CD1a and CD207 (Langerin) is considered the immunophenotypic hallmark of the LC [109, 125]. These cells are positive for CD4, S-100 protein, and HLA-DR and are negative for CD21 and CD35 (Figure 21.14). The LCH may aberrantly express CD52 [126]. The extracutaneous lesions appear to be consisting of less mature LCH cells, and in addition to CD1a and Langerin, express CD14 and CD68, whereas the skin lesions consist of more mature LCH and lack the expression of CD14 and CD68 [109]. CD45 and lysozyme are weakly positive and CD15, CD30, CD33, CD34, and MPO are negative [108, 109, 122, 123].

Molecular and Cytogenetic Studies

Monoclonal nature of LCH has been suggested by using X-linked polymorphic DNA probes [112]. There is an association between LCH and monosomy 7 [121, 122]. Loss of heterozygosity at 9p21, 17p, and 22q has been reported in patients with LCH [111, 127]. As mentioned earlier, several molecular cytogenetic abnormalities have been reported in patients with LCH, such as gains of DNA copy number on chromosomes 2q, 4q, and 12 and loss of DNA sequences on chromosomes 1p, 5, 6, 16, and 22q [121].

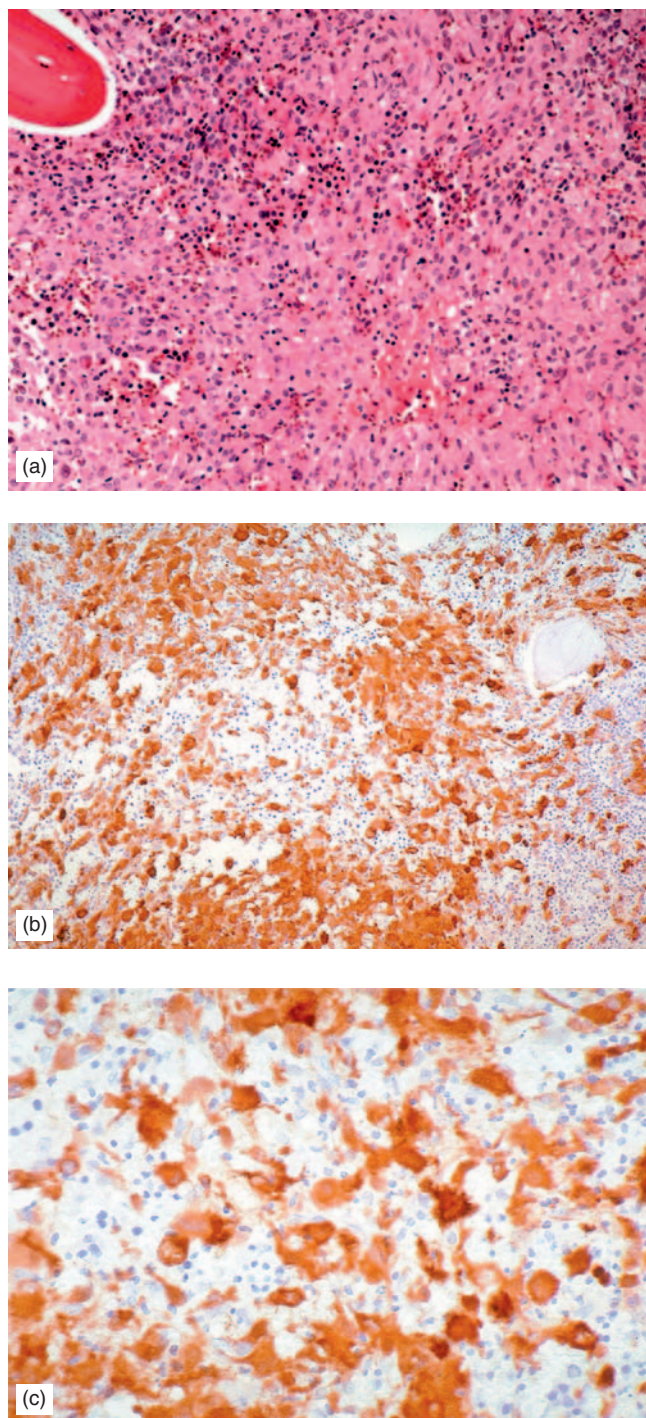


FIGURE 21.14 Langerhans cell histiocytosis. Bone marrow biopsy section demonstrates a diffuse infiltration by the Langerhans cells (a) H&E. These cells express S-100 (b) low power and (c) high power views, immunohistochemical stain.

Clinical Aspects

LCH is primarily a disease of childhood and early adulthood with a male:female ratio of 3–4:1. People from the northern Europe are more commonly affected [123]. The disease is rare in Afro-Americans. A wide spectrum of clinical manifestations have been described with their own specific terminology, ranging from congenital self-healing form

(Hashimoto–Pritzker disease) to solitary disease (eosinophilic granuloma) with good prognosis to multifocal multisystem disease with poor prognosis (Table 21.8).

Bone is the most frequent site of involvement, usually presenting as a lytic lesion of the skull, which may be painful or asymptomatic [128]. Other frequent sites of bone involvement include femur, ribs, vertebra, and humerus. The skin lesions in infants appear as brown to purplish papules and may mimic congenital neuroblastoma or leukemia [123, 129]. Hepatosplenomegaly and lymphadenopathy are the major clinical presentations of the multisystem LCH. Lung lesions are associated with heavy smoking and are frequently observed in young adults. There is an association between multisystem LCH and acute lymphoblastic leukemia [5].

Different therapeutic approaches have been proposed. Recommendations for unifocal osseous lesions include surgical curettage or excision, radiation or single agent chemotherapy, or combination of all. For pediatric multisystem LCH, combination chemotherapy, such as LCH-1 protocol (vinblastine versus etoposide in combination with intravenous steroids), LCH-2 protocol (vinblastine, oral prednisone, and mercaptopurine with or without etoposide), and LCH-3 protocol (vinblastine and prednisone with or without methotrexate), is recommended [130–132].

Differential Diagnosis

The differential diagnosis includes a garden variety of histiocytic disorders such as lysosomal storage diseases, HLH, and granulomatous disorders. Hepatosplenomegaly and lymphadenopathy may raise the possibility of leukemia/lymphoma. Expression of CD1a and Langerin and the presence of ultrastructural Birbeck granules are diagnostic features for LCH. LCH is distinguished from LC sarcoma by the lack of atypical cytologic features such as pleomorphic, hyperchromatic nuclei, prominent nucleoli, or numerous mitotic figures (see the following section).

Langerhans Cell Sarcoma

Langerhans cell sarcoma is a rare neoplasm of LC characterized by explicit malignant cytologic features (Figure 21.15) [5, 133, 134]. These features include hyperchromatic and pleomorphic nuclei, prominent nucleoli, and high mitotic figures (usually >5/hpf). Associated inflammatory cells, such as eosinophils, are lacking or minimal [5, 134]. The neoplastic cells in most cases, similar to the LCH, express CD1a, Langerin, and S-100 protein and demonstrate the ultrastructural Birbeck granules. They may also aberrantly express CD31 or CD56 [125, 135]. The Ki-67 index is usually high. Langerhans cell sarcoma has been reported in both children and adults. It is characterized by multisystem involvement and poor prognosis. The involved organs include bone, skin, spleen, liver, lymph nodes, and lung [135].

Agranular CD4+, CD56+ Hematodermic Neoplasms (Blastic NK-Cell Lymphoma)

This entity is called “Blastic NK-cell lymphoma” in the WHO classification and is defined as a lymphoid malignancy

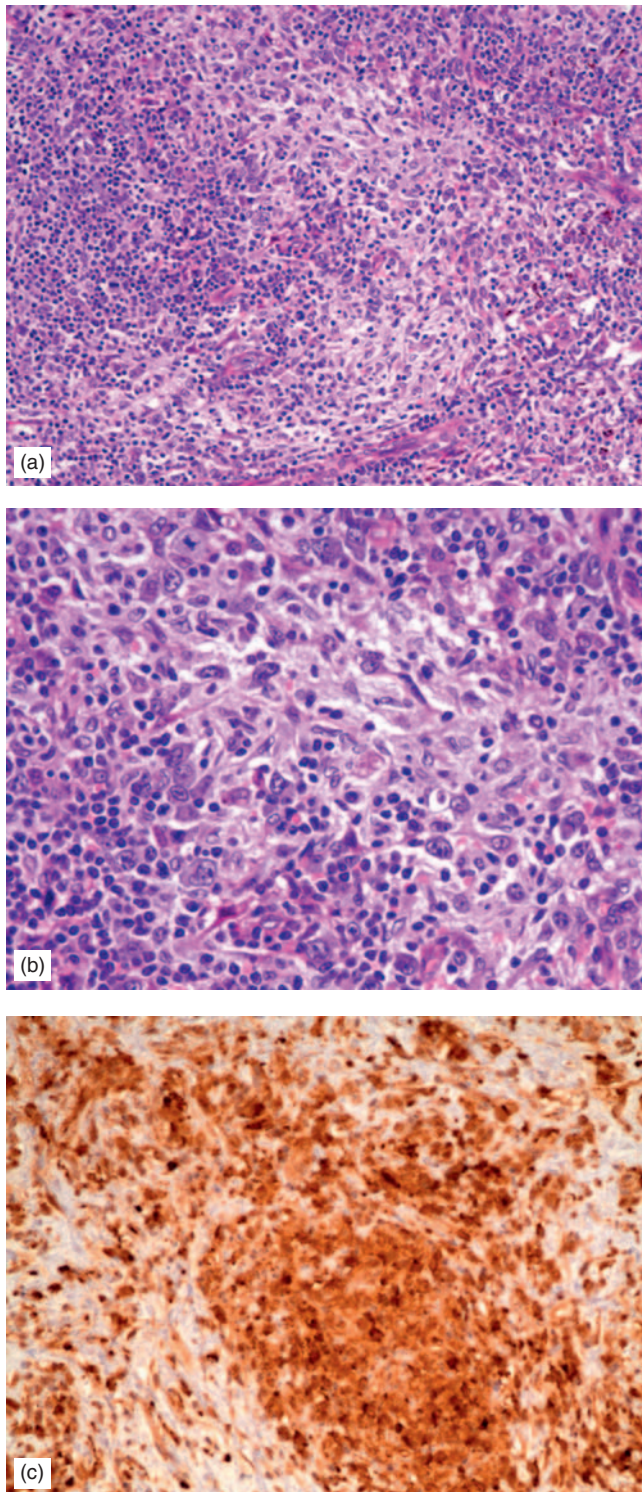


FIGURE 21.15 Lymph node section demonstrating clusters of large, atypical pleomorphic cells that were positive for CD1c and S-100. The lesion may represent Langerhans cell sarcoma. (a) and (b) are low and high power views, (c) represents immunohistochemical stain for S-100.

consisting of blast cells expressing NK-cell-associated marker, CD56 [5]. However, more recent studies suggest that these tumors originate from plasmacytoid dendritic cells [136–139].

Etiology and Pathogenesis

The etiology and pathogenesis are not known. There is no evidence of a viral etiology.

Pathology

Morphology

The skin involvement is usually multifocal, often with formation of nodules (Figure 21.16). Large clusters of monomorphous cells are present in the dermis and the hypodermis with infiltration of the cutaneous appendages. A single-file infiltration may be present in some areas. The epidermis is spared and there is no evidence of angiocentrism or angiodestruction [137].

The neoplastic cells are blastic with variable amount of weakly basophilic, non-granular cytoplasm with round, oval, or cleaved nuclei, fine chromatin, and multiple prominent nucleoli (Figure 21.17). The cytoplasm may show peripheral microvacuoles or pseudopods. The tumor cells may vary in size from small to large and in some cases consist of a mixture of small and large blastic cells. The blood, bone marrow, and lymph nodes are other frequent sites of involvement.

The bone marrow is involved in >80% of the cases showing focal or diffuse infiltration by the blastic tumor cells. Circulating blast cells are detected in about 60% of the patients ranging from 1% to >90% of the leukocyte counts [140]. The affected lymph nodes usually show a leukemic pattern of infiltration with the involvement of medulla, sinuses, and interfollicular areas.

Immunophenotype

The major immunophenotypic features of the hematodermic neoplasms are presented in Table 21.9. These cells characteristically express CD4, CD56, CD43, CD45(dim to strong), TCL1, HLA-DR, and CD123. CD68 is positive in about 50% and TdT is positive in about 25% of the cases (Figure 21.18). The overwhelming majority of cases are negative for other T- and NK-cell markers and B-cell- and myeloid-associated antigens. Most of these immunophenotypic features (coexpression of CD4, CD43, CD45, TCL1, HLA-DR, CD123, and CD68) along with the exception of CD56 are shared with the plasmacytoid dendritic cells, suggesting a lineage relationship [136–139].

Molecular and Cytogenetic Studies

Molecular studies have not shown TCR rearrangements in these neoplasms. Approximately 65% of cases are associated with cytogenetic abnormalities, which are often complex and with an aneuploid karyotype [137, 141]. Six major recurring abnormalities have been reported: deletions of 12p13 and 6q23, monosomy 9, monosomy 15, and abnormalities of chromosomes 5 (5q21 and 5q34) and 13 (between 13q13 and 13q21) (Figure 21.19) [141].

Clinical Aspects

Hematodermic neoplasm is a rare disease affecting patients at any age, ranging from 8 to 96 years, with a median age of 65 years [137, 140, 142]. The most frequent

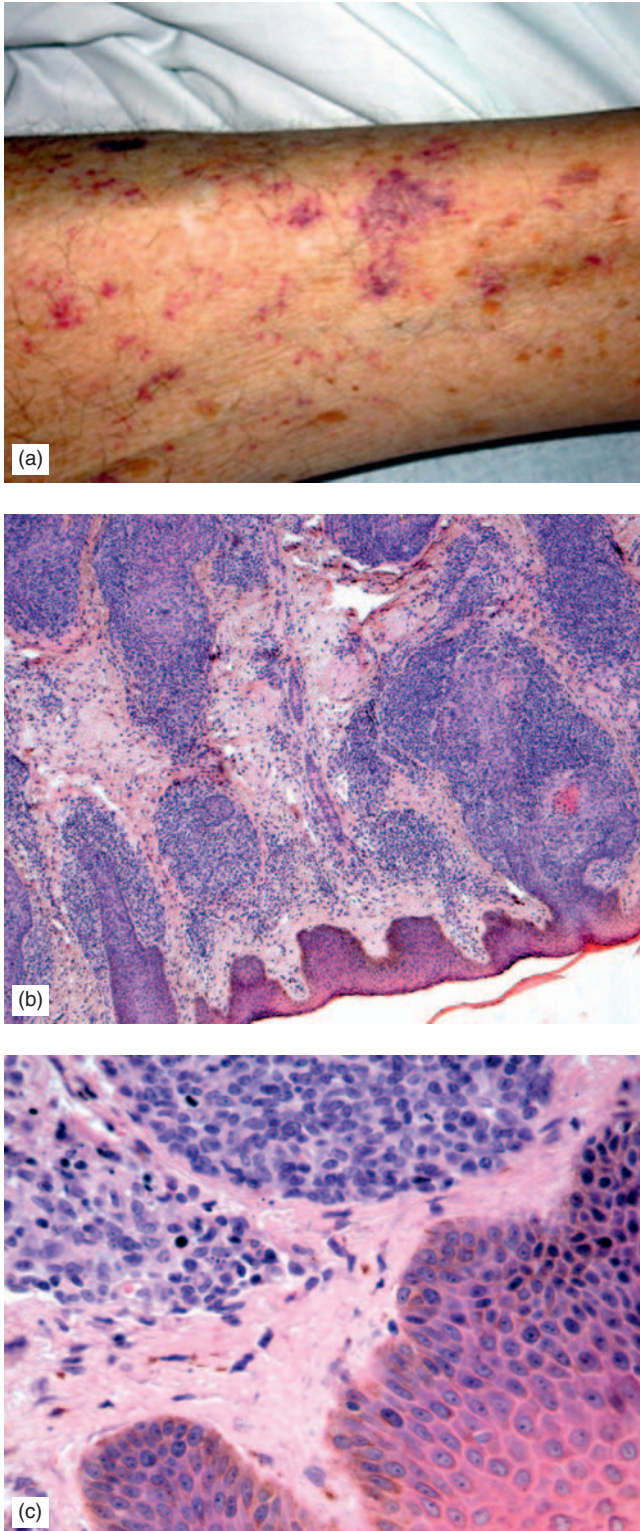


FIGURE 21.16 Agranular CD4+ , CD56+ hematodermic neoplasm. Skin demonstrates multifocal lesions in various sizes and shapes (a) with heavy infiltration of the neoplastic cells in the dermis and surrounding skin appendages (b). High power view shows immature mononuclear cells with oval or irregular nuclei, fine chromatin, and prominent nucleoli (c).

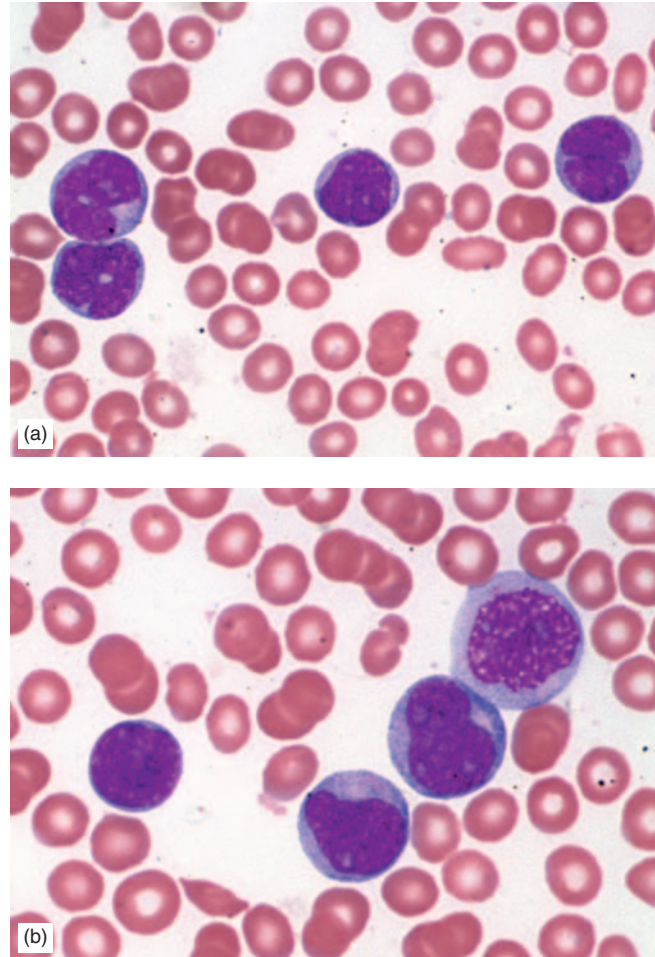


FIGURE 21.17 Blood smear from a patient with hematodermic neoplasm demonstrates numerous immature mononuclear cells in various sizes and shapes (a and b). Some of the immature cells mimic monoblasts.

site of involvement is skin that is affected in >90% of the patients [137, 143–145]. The cutaneous lesions are usually purple and solitary or localized at the beginning but become multiple with time. They may appear as nodules or patches. A leukemic presentation with circulating blasts is reported in about 60%, and splenomegaly and/or lymphadenopathy are reported in about 60% of the cases [140, 141]. Other sites of involvement include the liver, lung, and central nervous system [140].

Combination chemotherapy may achieve complete remission, but in most patients, the disease relapses between 3 and 18 months. The overall reported 2-year survival rate is about 25% [140].

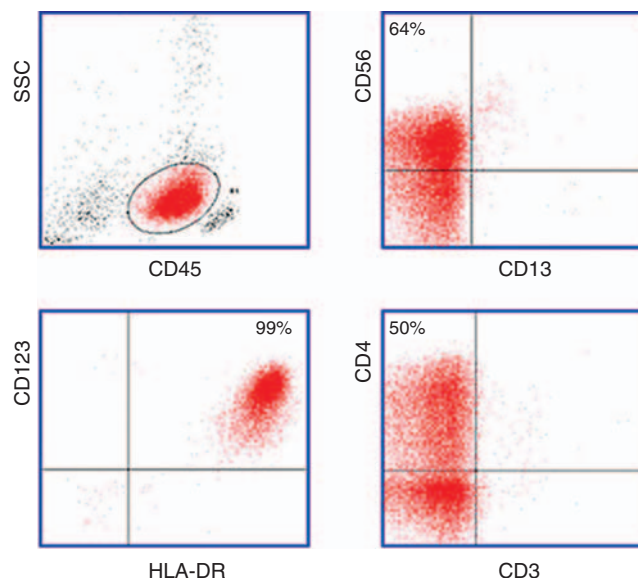
Differential Diagnosis

The differential diagnosis includes primary and secondary cutaneous lymphomas/leukemias such as nasal type T/NK-cell lymphoma, aggressive NK-cell leukemia/lymphoma, and mycosis fungoides. The main distinguishing features between the hematodermic neoplasm and the cutaneous nasal type T/NK lymphoma are the lack of angiocentric and angiodestructive lesions, TIA-1 expression, and EBV genome

TABLE 21.9 Immunophenotypic features of hematodermic neoplasms*.

Markers	Results
CD4	+
CD56	+
CD123	+
CD43	+
CD45	+
HLA-DR	+
TCL1	+
CD68	±
CD7	±
CD2	±
CD36	±
CD38	±
TdT	±
CD1a	–
CD3	–
CD5	–
CD8	–
CD16	–
CD57	–
TIA-1	–
CD10	–
CD19	–
CD20	–
CD79a	–
CD13	–
CD14	–
CD15	–
CD33	–
MOP	–
CD117	–
CD34	–

*Adapted from Ref. [137].

**FIGURE 21.18** Flow cytometry of blood sample of a patient with agranular CD4+ , CD56+ hematodermic leukemia. Neoplastic cells express CD45^{dim}, CD123, HLA-DR, CD56 (partial), and CD4 (partial).

in the hematodermic neoplasms. The NK-cell tumors often show cytoplasmic granules and are mostly CD4–, CD8+, and TIA-1+, whereas hematodermic neoplasms are CD4+, CD8–, and negative for TIA-1. Mycosis fungoides is distinguished from hematodermic neoplasm by epidermal involvement (Pautrier abscesses) and CD3+, CD56– immunophenotype.

Also, cutaneous infiltrations of myelomonocytic leukemias may show significant overlapping of immunophenotypic features with hematodermic neoplasms by expressing CD4, CD56, CD68, and HLA-DR. However, TCL1 is expressed in 90% of the hematodermic neoplasms and <20% of the acute myeloid leukemias [137], and myeloid-associated markers, such as CD13, CD14, CD15, CD33, and CD117, are negative in hematodermic neoplasms (Table 21.9).

Interdigitating Dendritic Cell Tumor (Sarcoma)

Interdigitating dendritic cell tumor (sarcoma) is an extremely rare neoplasm consisting of cells with spindle-shaped nuclei and immunophenotypic features similar to the IDC [5, 146]. Lymphadenopathy is the most frequent clinical findings which may present as localized or generalized. Cases of extranodal involvement such as the skin, intestine, spleen, and soft tissues have been observed [5, 147–149]. This disorder has been reported in ages from 8 to 77 years, but most patients are adults with a median age of over 50 years [146, 150]. Clinical outcome is variable, and therefore, IDC *tumor* may be a more suitable term.

The involved lymph nodes often show paracortical infiltration of elongated cells with ovoid or spindle-shaped nuclei in a storiform pattern (Figure 21.20). These cells have a variable amount of cytoplasm with indistinct cell borders,

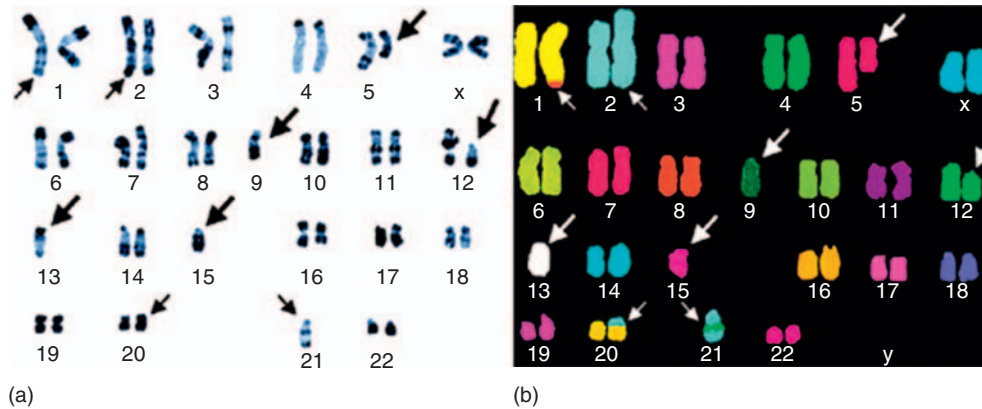


FIGURE 21.19 Agranular CD4+, CD56+ hematodermic leukemia. (a) Representative R-banded and (b) M-FISH-Metasystems probe karyotypes. Arrows indicate the presence of chromosomal abnormalities; large arrows identify recurrent anomalies. From Ref. [140] by permission. This research was originally published in *Blood*.

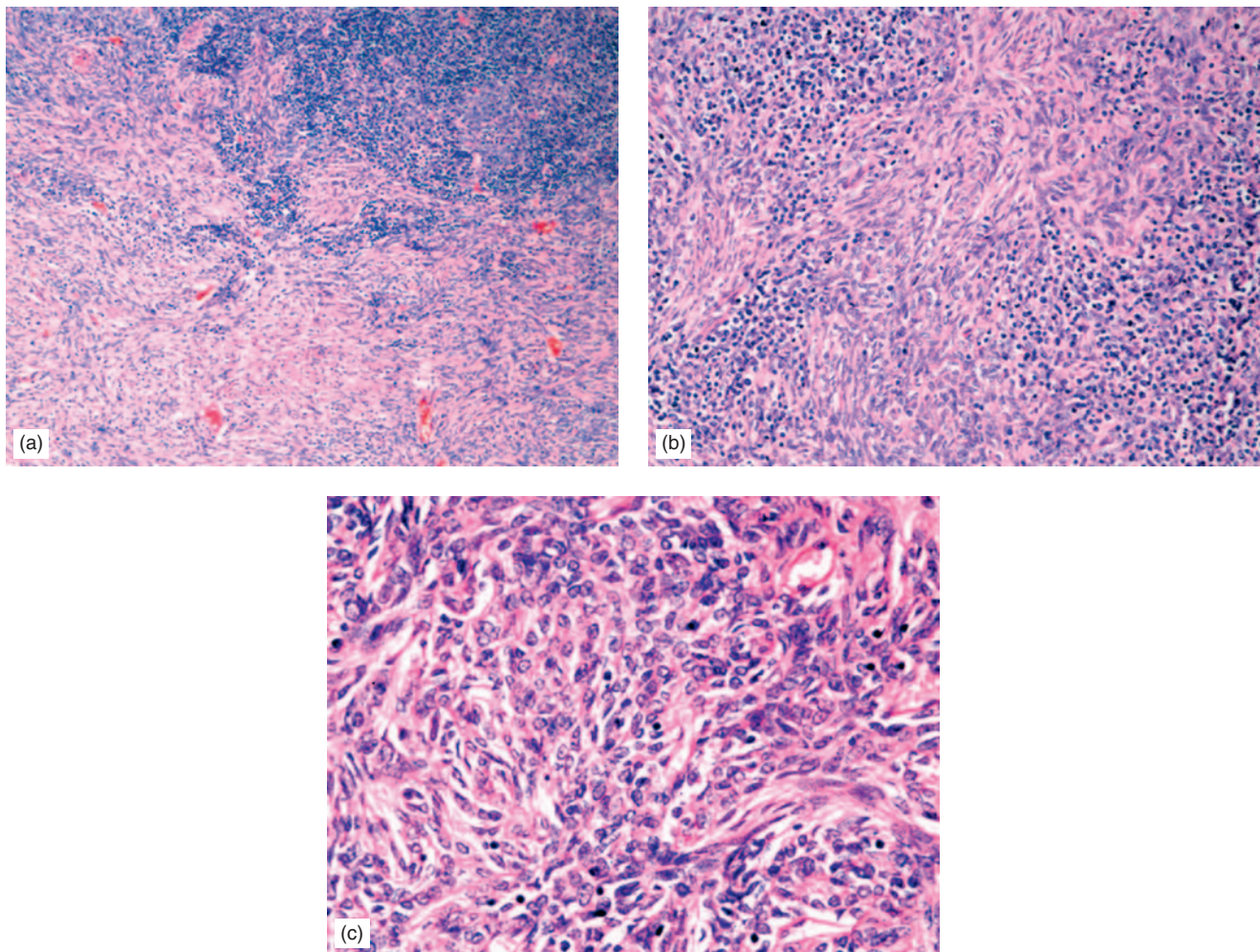


FIGURE 21.20 A lymph node-based lesion showing sheets of spindle cells in a storiform pattern, highly suggestive of interdigitating dendritic cell tumor at (a) low power, (b) intermediate power, and (c) high power views. Tissue blocks were not available for immunohistochemical stains.

a vesicular nuclear chromatin, and a distinct nucleus [5, 146]. A variable degree of cytologic atypia may be present. Mitotic figures are usually low ($<5/\text{hpf}$) and there is lack of necrosis. Residual follicular structures are often present [5].

Immunophenotypic characteristics are the expression of S-100 protein, Vimentin, and HLA-DR and the lack of expression of CD1a, CD21, CD35, and pan-B- and pan-T-cell markers (Table 21.1). The neoplastic cells may show weak positive reactions for CD45, CD68, or lysozyme [5, 147–149].

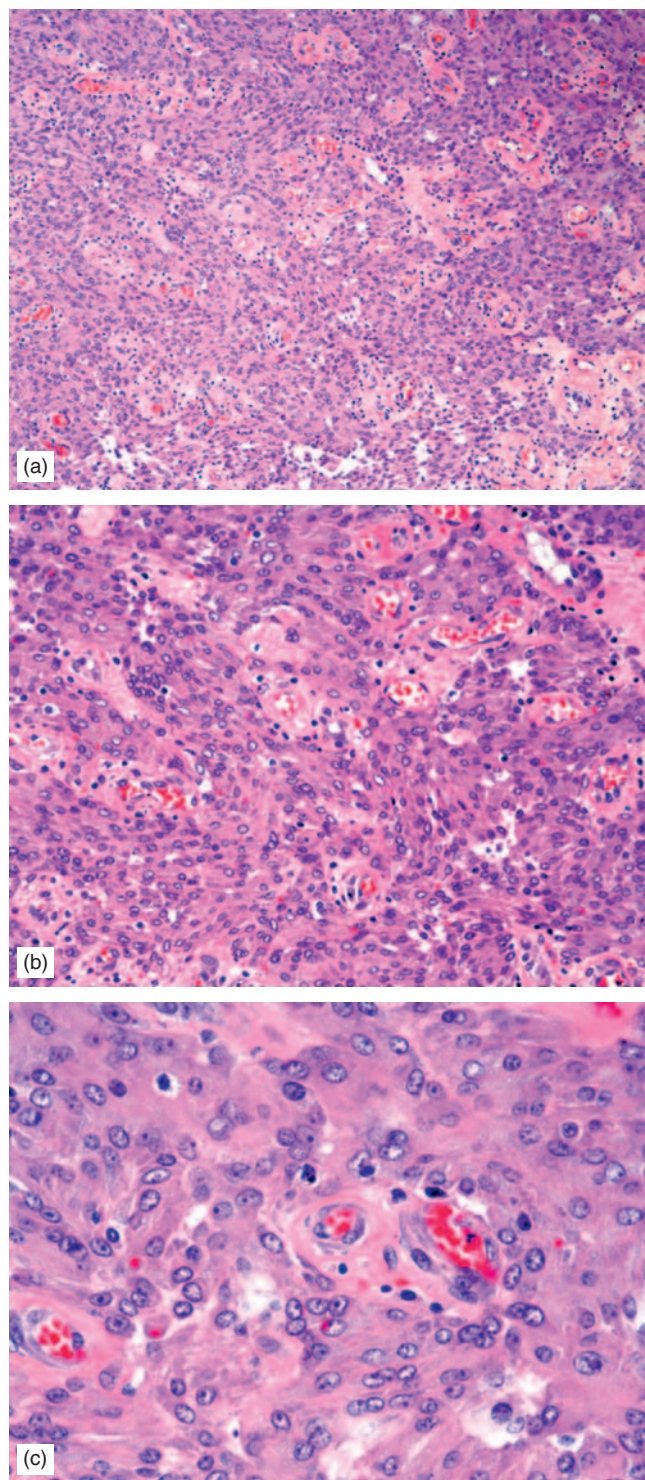


FIGURE 21.21 Follicular dendritic cell tumor. Sheets of tumor cells with spindle to oval nuclei and relatively abundant cytoplasm in a vascular stroma: (a) low power, (b) intermediate power, and (c) high power views. Slide from CAP, PIP-D 2007, Case # 2007-33. Neoplastic cells were reported to be positive for CD21, CD35, and clusterin.

They are negative for CD30, CD34, epithelial membrane antigen (EMA), and myeloperoxidase (MPO). Ki-67 is expressed in about 10–20% of the tumor cells. The immunophenotypic features are helpful in distinguishing these tumors from the follicular dendritic neoplasms and other sarcomas.

Follicular Dendritic Cell Tumor (Sarcoma)

Follicular dendritic cell tumor (sarcoma) is an extremely rare neoplasm consisting of cells with spindle-shaped nuclei and immunophenotypic features similar to the FDC [5, 151–153]. Lymph nodes are the most frequent sites of involvement [152, 153]. Extranodal involvement has been reported in about 25% of the cases, primarily affecting intra-abdominal organs [153–155]. It is a disease of young and middle-aged adults, usually with an indolent clinical course, high recurrent rate, and low risk of metastasis [5, 155]. For these reasons, FDC *tumor* appears to be a more suitable term.

The neoplastic cells have a variable amount of cytoplasm with indistinct cell borders, a vesicular nuclear chromatin, and a distinct nucleus (Figure 21.21) [5, 155]. A variable degree of cytologic atypia and occasional multinucleated giant cell may be present. Mitotic figures are usually $\leq 10/\text{hpf}$, but occasional cases with $>30/\text{hpf}$ have been reported [5]. The neoplastic cells form fascicles and whorls and/or demonstrate a storiform pattern. Residual follicular structures or lymphoid tissues are often present [5].

Immunophenotypic features include the expression of CD21, CD23, and CD35 as well as HLA-DR and Vimentin. The tumor cells may variably express S-100 protein, EMA, and CD68 [152, 154]. Ki-67 staining is usually $\leq 25\%$ [5]. CD1a, CD30, CD34, cytokeratin, and lysozyme are negative. The immunophenotypic features are helpful in distinguishing these tumors from the interdigitating dendritic neoplasms and other sarcomas.

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Granulocytic Disorders

Faramarz Naeim

This chapter is devoted to the qualitative and quantitative non-neoplastic granulocytic disorders or granulocytic abnormalities associated with primary bone marrow disorders. It includes topics such as morphologic and functional abnormalities, neutropenia, neutrophilia, eosinophilia, and basophilia.

MORPHOLOGIC ABNORMALITIES

Toxic Granulation

This term is used to describe the presence of purple to dark-blue granules, resembling primary granules in segmented neutrophils, bands, and metamyelocytes (Figure 22.1). They represent lysosomal granules, contain myeloperoxidase (MPO), and show increased alkaline phosphatase activity. They indicate a shortened maturation time and activation of post-mitotic neutrophilic pool. They are found in infections and acute inflammations. They are often associated with Dohle bodies.

Dohle Inclusion Bodies

Dohle bodies are round, oval, elongated, or triangular, blue, gray-blue, or gray-green cytoplasmic inclusions (Figure 22.1b). They are RNA-contacting structures apparently derived from rough endoplasmic reticulum [1, 2]. Dohle bodies are found in activated post-mitotic neutrophilic series, including metamyelocytes, bands, and segmented neutrophils. They are frequently associated with toxic granulation

and are found in infections, burns, traumas, and drug toxicity [1, 3, 4].

May-Hegglin Anomaly

May-Hegglin anomaly is a hereditary condition in which Dohle body-like inclusions are found in neutrophils, eosinophils, basophils, and monocytes (Figure 22.2). May-Hegglin anomaly is a member of a group of rare, autosomal dominant disorders characterized by thrombocytopenia, giant platelets, and Dohle body-like inclusions. It is associated with mutations in the *MYH9* gene mapped to chromosome 22q12.3-q13.2 [5–9]. It represents variable clinical manifestations including sensorineural deafness, cataracts, and nephritis.

Alder-Reilly Anomaly

Alder-Reilly anomaly is characterized by the presence of dense azurophilic granules (resembling toxic granules) in neutrophils, eosinophils, basophils, and, sometimes, lymphocytes and monocytes (Figure 22.3). This anomaly is seen in patients with mucopolysaccharidosis (Hurler's and Hunter's syndromes) and myelodysplastic syndromes [10–12]. Alder-Reilly anomaly has been reported in association with a mutation of the MPO structural gene [12].

Chediak-Higashi Granules

Chediak-Higashi syndrome (CHS) is a rare autosomal recessive disorder characterized by severe immune deficiency, partial albinism, bleeding tendencies, and recurrent bacterial

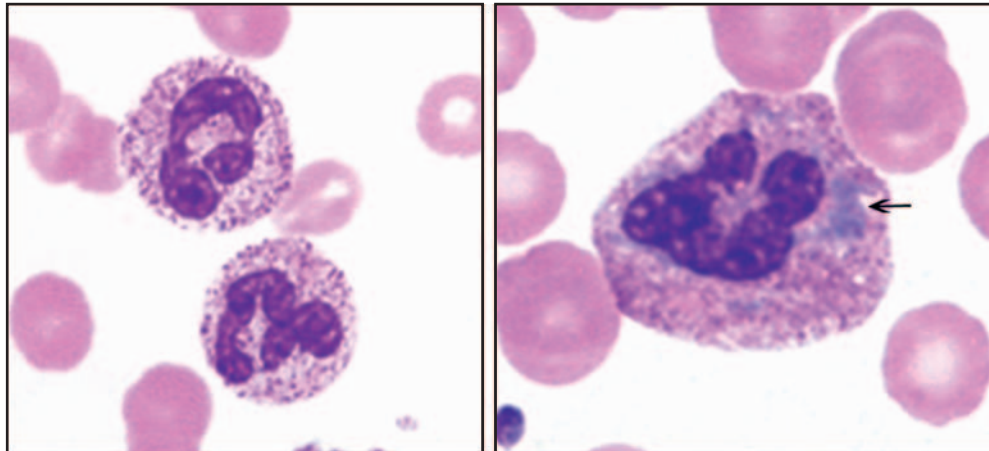


FIGURE 22.1 Toxic granulation and Dohle body (arrow) in neutrophils.

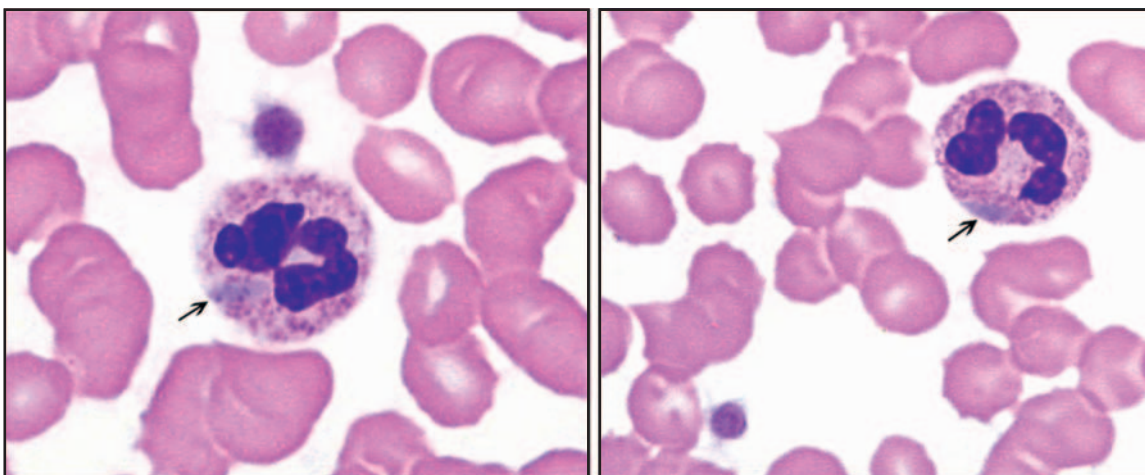


FIGURE 22.2 Neutrophils showing Dohle body-like inclusions (arrows) in May–Hegglin anomaly.

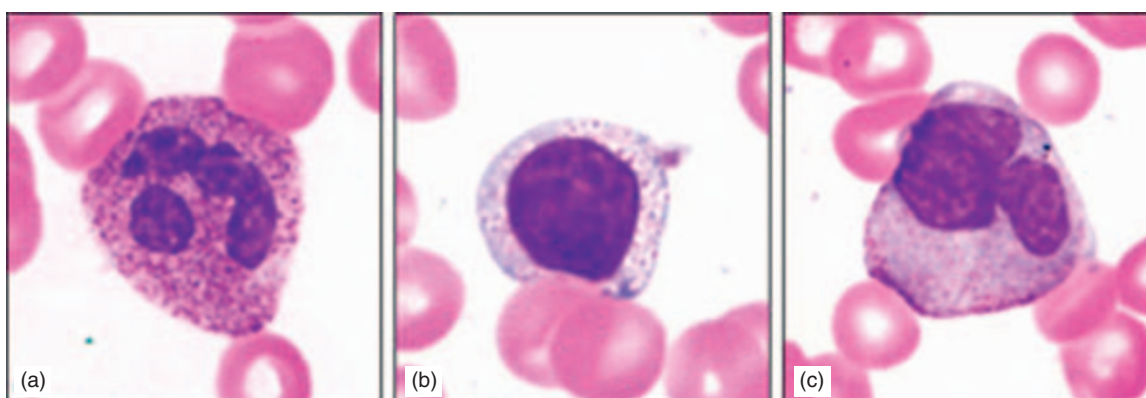
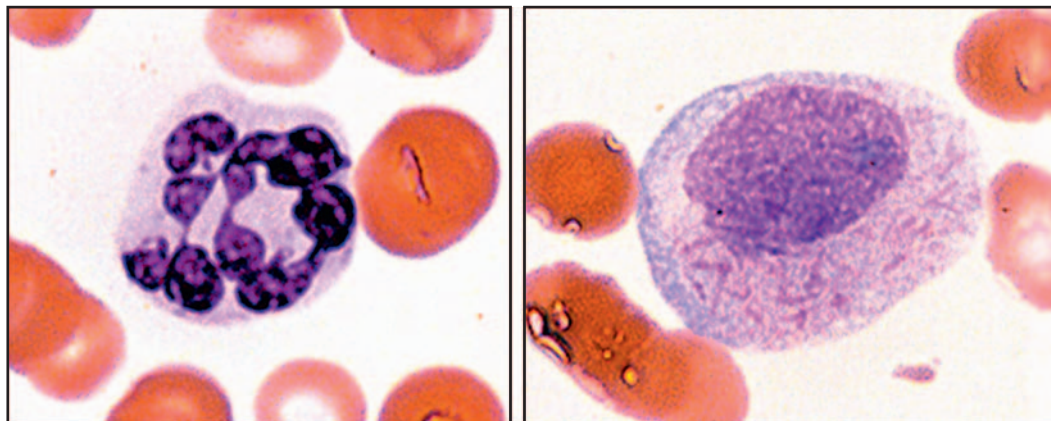


FIGURE 22.3 Alder–Reilly anomaly is characterized by the presence of dense azurophilic granules (resembling toxic granules) in neutrophils (a), lymphocytes (b), and monocytes (c).

**FIGURE 22.4**

A hypogranular, hypersegmented neutrophil is shown on the left and a late promyelocyte loaded with Auer rods is depicted on the right.

infections [13, 14]. The morphologic hallmark of CHS is the presence of giant, blue or greenish gray cytoplasmic granules in various cells, including monocytes, granulocytes, cytotoxic T-cells, NK-cells, melanocytes, and Schwann cells [13, 14] (see Chapter 21).

Auer Rods

Auer rods are rod-like cytoplasmic inclusions found in myeloid precursors in patients with acute myelogenous leukemia (Figure 22.4). They are formed from the fusion of primary granules and, therefore, are MPO-positive.

Absence or Reduction of Cytoplasmic Neutrophilic Granules

Absence or reduced cytoplasmic granules are characteristic features of granulocytic cells in myelodysplastic syndrome (Figure 22.4). Selective defect in lactoferrin gene expression has been reported in association with neutrophil-specific granule deficiency [15].

Atypical Eosinophils and Eosinophilic Granules

In certain conditions, such as a subtype of acute myelomonocytic leukemia with inversion of chromosome 16(p13q32) and rare cases of chronic myelogenous leukemia, eosinophils contain atypical basophilic granules (Figure 22.5) [16, 17]. In hypereosinophilic syndrome, eosinophils may show hypersegmentation (Figure 22.5, inset).

Abnormal Nuclear Morphology

Pelger–Huet anomaly is an autosomal dominant disorder characterized by defective nuclear segmentation (hyposegmentation) in neutrophils (Figure 22.6) [18–20]. An

association has been reported between this entity and *LBR* (lamin B-receptor) gene mutation located at chromosome 1q41–43 [21, 22]. The acquired hyposegmentation of the neutrophil nucleus, also called pseudo-Pelger–Huet anomaly, has been observed in sepsis, myelodysplastic syndromes, myeloproliferative disorders, some leukemias/lymphomas, and patients with solid organ transplantation [23–25].

Neutrophil nuclear hypersegmentation (more than five lobes) is observed in megaloblastic anemia, chronic infection, myelodysplastic syndrome, iron deficiency, hypogonadism, and chronic myelogenous leukemia (Figure 22.7) [3, 26, 27]. Neutrophilic hypersegmentation has been reported in two siblings, suggesting an autosomal recessive trait [28].

FUNCTIONAL ABNORMALITIES

Functional abnormalities of granulocytes are of two major types: (1) intrinsic defects, such as chemotactic disorders, CHS, adhesion defects, and MPO deficiency, and (2) extrinsic disorders, such as abnormalities of opsonizing systems due to abnormal and/or complement defects. CHS demonstrates both morphologic and functional abnormalities in a variety of hematopoietic and non-hematopoietic cells (see Chapter 21). In this section, the following intrinsic defects are briefly discussed: MPO deficiency, chronic granulomatous disease (CGD), and leukocyte adhesion deficiency (LAD).

MPO Deficiency

MPO deficiency is the most common intrinsic granulocytic functional deficiency [29–33]. It is an autosomal recessive inherited disorder involving the *MPO* gene located at 17q23 [34, 45]. MPO plays a critical role in the microbicidal activity of neutrophils. Activated neutrophils release MPO into the phagolysosomes or the extracellular spaces [34]. MPO, hydrogen peroxide, and chloride ion make up the cytotoxic mediators in infections [31, 32].

A secondary type of MPO deficiency has been observed in patients with clonal hematopoietic disorders such as

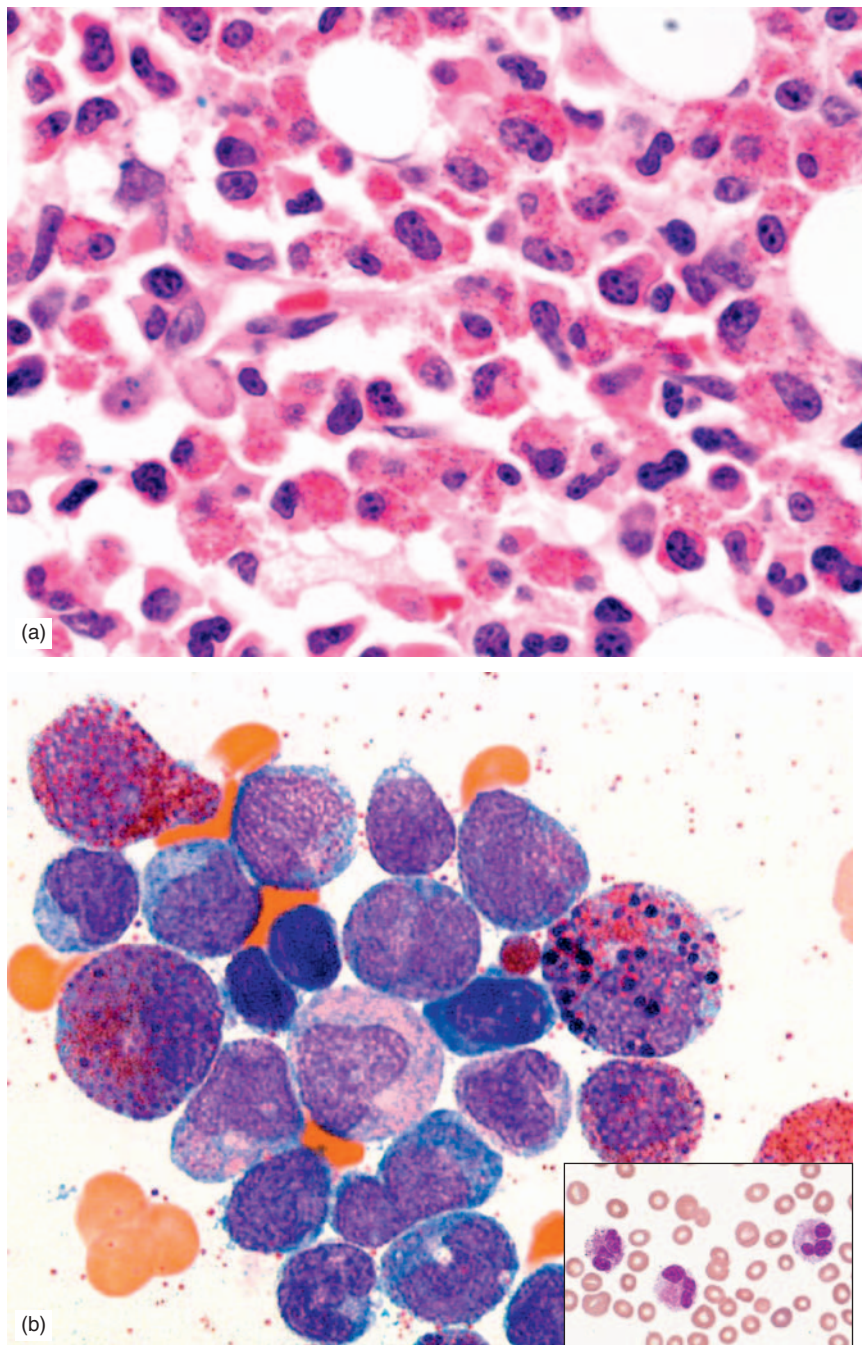


FIGURE 22.5 Bone marrow biopsy section (a) shows sheets of eosinophils. Bone marrow smear (b) demonstrates eosinophilic precursors with basophilic granules and blood smear (inset) shows eosinophils with hypersegmented nuclei.

myelodysplastic syndrome, acute myeloid leukemia, and lymphoma. The MPO deficiency in these patients is often due to discrete chromosomal aberrations involving the *MPO* gene [35].

Chronic Granulomatous Disease

CGD is characterized by the deficiency of respiratory burst oxidase and inability to manufacture superoxide (O_2^-) for the formation of microbicidal oxidants [36, 37]. CGD is an inherited disorder. It is either X-linked or autosomal recessive [38, 39]. The X-linked CGD almost exclusively involves men, except in rare homozygosity conditions or when there

is a coexistent inactivated normal gene. The autosomal-recessive CGD occurs with equal frequency in male and female.

Neutrophils and other phagocytes (such as eosinophils and monocytes) use an NADPH oxidase to generate superoxide. The NADPH oxidase consists of five subunits. Two subunits, gp91-PHOX and p22-PHOX, form the heavy and light chains of the cytochrome b_{558} (CYBB) and are membrane-associated (membranes of secretory vesicles and granules) [40]. The three remaining subunits, p40-PHOX, p47-PHOX, and p67-PHOX, are cytosolic components. The gene encoding gp91-PHOX is located on X-chromosome. Mutation of this gene occurs in approximately 70% of the patients with CGD [40]. The remaining 30% of the cases

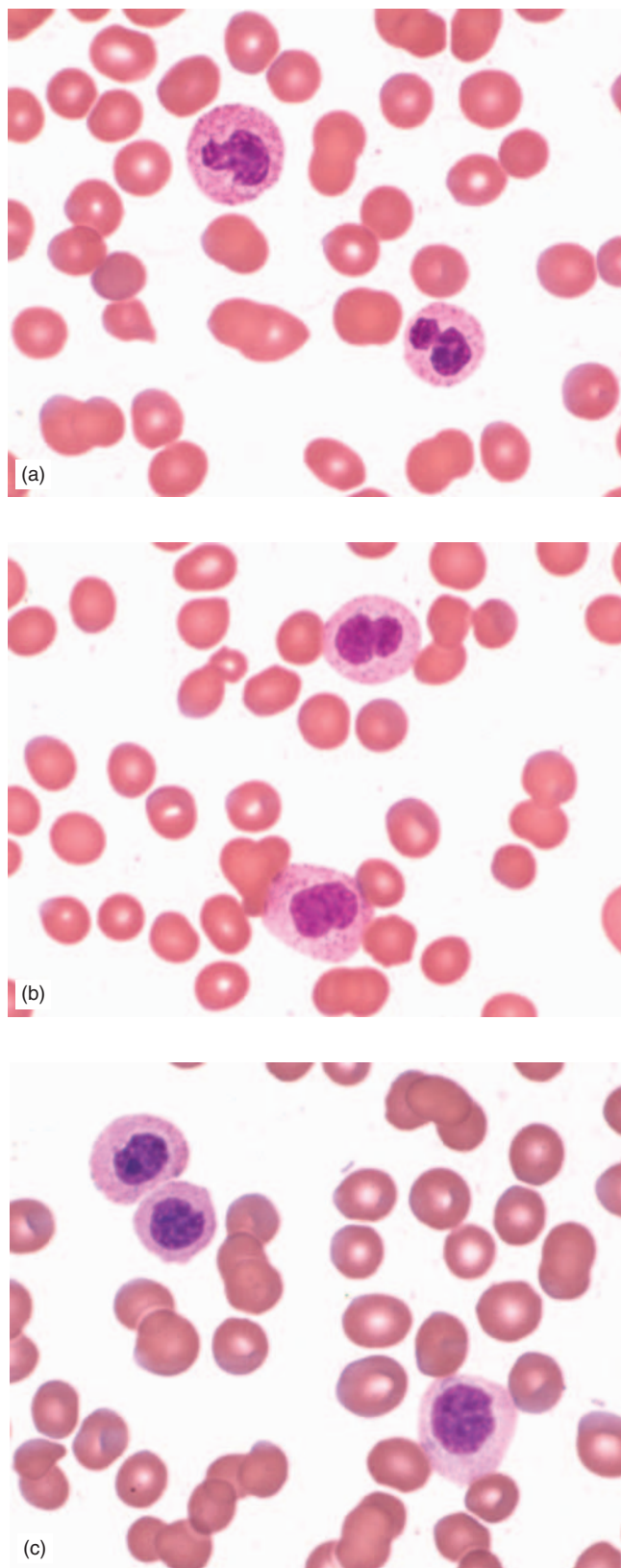


FIGURE 22.6 Blood smears (a, b, and c) demonstrate Pelger–Huet anomaly characterized by defective nuclear segmentation (hyposegmentation) in neutrophils.

are caused by mutations of autosomal genes encoding p22-, p47-, and p67-PHOX subunits.

Patients with CGD suffer recurrent bacterial and fungal infections often presenting as pneumonia, abscesses (skin, soft tissues, and organs), suppurative lymphadenitis, osteomyelitis, bacteremia, and fungemia. The severity of infection and the recurrence rate are variable, and the onset of clinical manifestations may range from early infancy to adulthood. The X-linked variant is seen in the younger population with a mean age of 3 years compared to about 8 years for the autosomal recessive forms [34]. The diagnosis of CGD is based on the demonstration of defective respiratory burst oxidase in neutrophils. The conventional laboratory test is the nitroblue tetrazolium (NBT) test, in which the oxygen produced in the course of a respiratory burst reduces the yellow, water-soluble tetrazolium dye to an insoluble deep-blue pigment. In normal conditions, at least 95% of neutrophils show positive reaction. The NBT test should be confirmed by DNA sequencing of the patient's *PHOX* genes [40].

Leukocyte Adhesion Deficiency

LAD syndromes are autosomal recessive disorders characterized by defective adhesion, binding and/or rolling of the leukocytes on the sites of microbial invasion [41]. These adhesion/rolling defects are of three major types: LAD I, LAD II, and LAD III [42, 43] (Figure 22.8).

LAD I syndromes are caused by impairment of expression of the leukocyte adhesion molecules (integrins). Integrins represent a family of glycoproteins, each composed of α - and β -subunits (CD11/CD18). The α -subunit, CD11, represents three different types of glycoprotein, CD11a, CD11b, and CD11c, respectively, for LFA-1 (expressed on all leukocytes), glycoprotein Mac-1, and p150/90 (both are expressed on monocytes, neutrophils, and NK-cells). The β -subunit, CD18, is shared in all integrins.

The LAD I is relatively rare. Its clinical outcome is often severe and includes recurrent or unresolved localized or systemic infections. The frequently reported clinical manifestations are delayed separation of the umbilical cord, recurrent bacterial infections, periodontitis, absence of pus formation, and impaired wound healing [41, 44]. Due to the lack of adhesion molecules and impaired mobilization of the leukocytes into the extravascular sites, these patients often have marked peripheral blood leukocytosis (5–20 times normal values) [43]. The diagnosis is established by flow cytometry demonstrating lack or marked reduction of the expression of integrins (CD11/CD18). DNA sequencing will identify relevant gene mutations. Mild to moderate cases are treated by antibiotics, and severe cases require bone marrow or stem cell transplantation.

LAD II syndromes are extremely rare and are caused by defective fucosylated carbohydrate ligands for p-selectin glycoprotein-1 (PSGL-1) (Figure 22.9) [42, 45]. The primary defect is in fucosylation of macromolecules, particularly at the stage of transport of fucose to the Golgi apparatus. This defect results in the lack of expression of certain glycans, such as CD15a (SLe^x) and H-antigen (Bombay blood group) in these patients [43].

The LAD II patients have less severe and fewer infection episodes than the LAD I patients. This syndrome has been

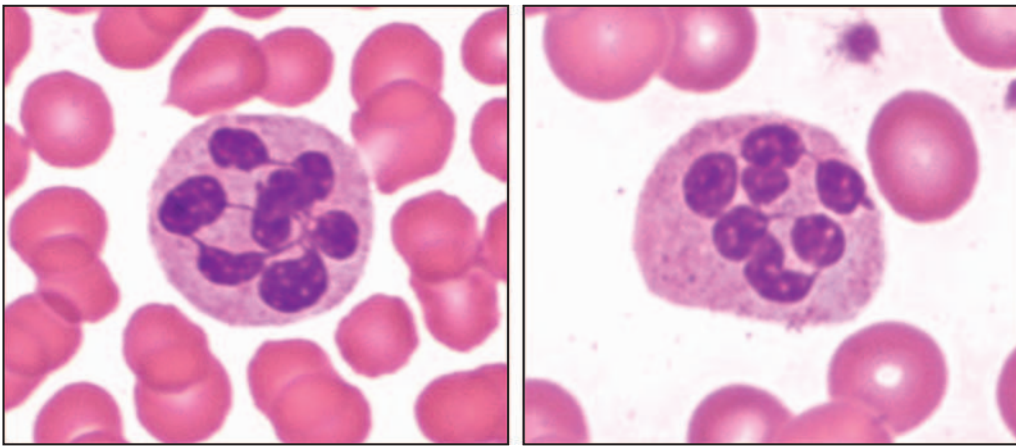


FIGURE 22.7 Neutrophil nuclear hypersegmentation (more than five lobes) is observed in megaloblastic anemia and other conditions such as chronic infection, myelodysplastic syndrome, hypogonadism, and chronic myelogenous leukemia.

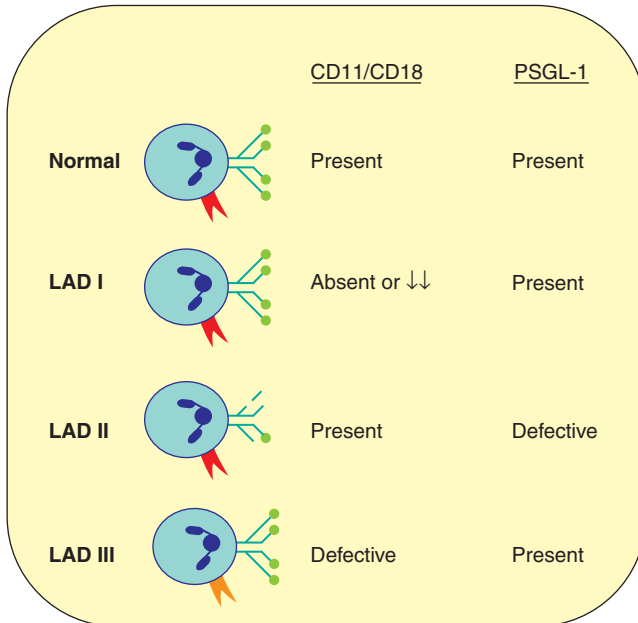


FIGURE 22.8 Altered surface phenotype in leukocyte adhesion deficiency. Expression of CD11/CD18 integrins and p-selectin glycoprotein 1 in various types of leukocyte adhesion deficiencies. Adapted from Ref. [41].

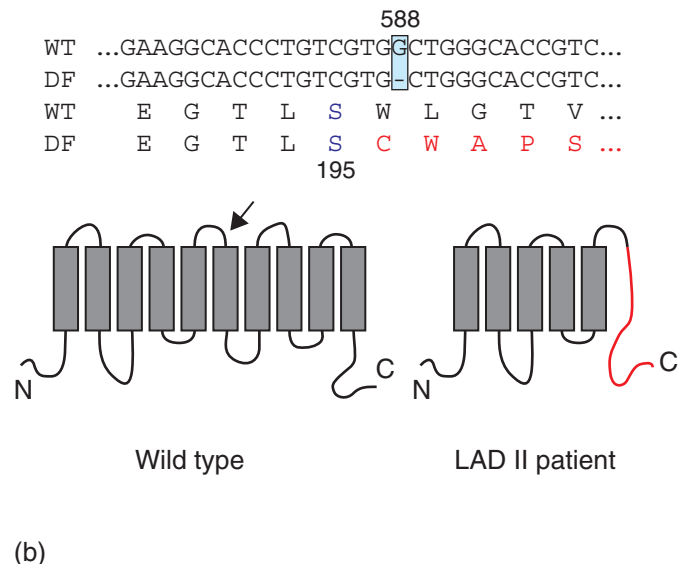
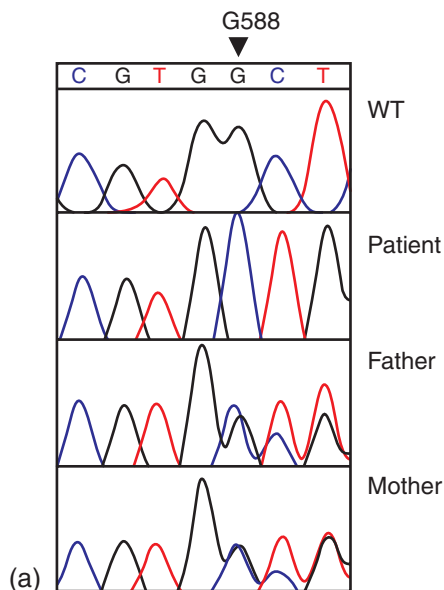


FIGURE 22.9 A single nucleotide deletion in position 588 (G588) in the *GDP-fucose transporter* gene of a patient with LAD II. (a) Chromatograms show a partial sequence for the *GDP-fucose transporter* gene from a healthy donor, the LAD II patient, and both parents. Note that G588 is absent in the patient, while both parents are heterozygotes in this position (overlaid sequence). (b) Partial primary sequence of the gene and the predicted protein region in which the deletion is found. A shift in the open-reading frame alters the protein sequence after Ser195 (arrow), as shown in the schematic representation of the predicted structure of the transporter. Adapted from Ref. [46] by permission. This research was originally published in *Blood*.

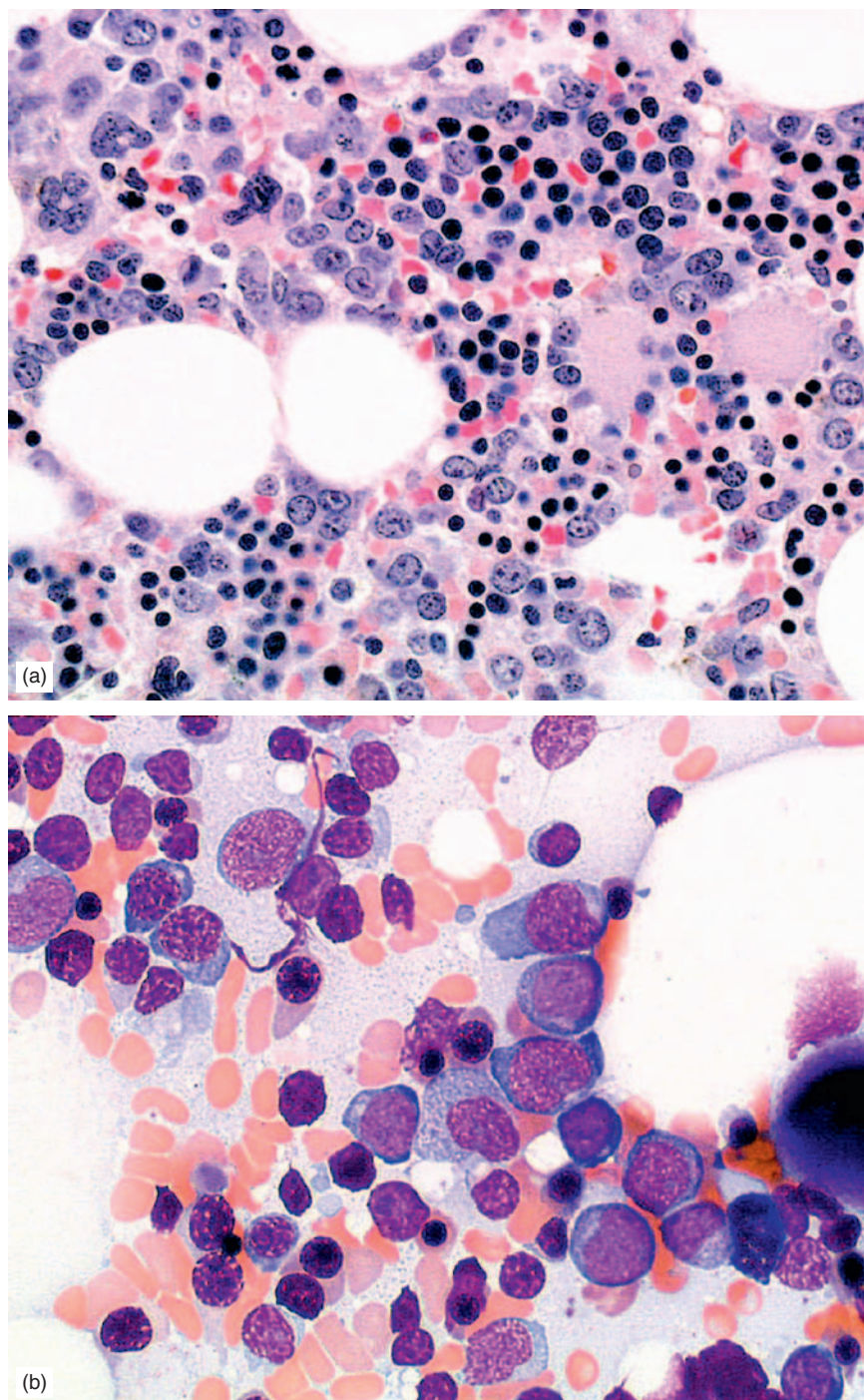


FIGURE 22.10 Agranulocytosis. Bone marrow biopsy section (a) and smear (b) demonstrate absence of late-stage granulocytic cells and sparse immature myeloid forms.

associated with mental retardation, short stature, microcephaly, depressed nasal bridge, and delayed motor development in affected children [43, 45, 46]. Other findings are leukocytosis (mainly neutrophilia) and Bombay blood group. The CD15a expression is lacking on the leukocytes by flow cytometry.

LAD III (previously called LAD I variant) is extremely rare and is characterized by a defect in integrin activation. The structure and expression of CD11 and CD18 appear to be intact [47]. Clinical symptoms are similar to those of LAD I. The prognosis is poor, and bone marrow transplantation is the treatment of choice.

NEUTROPENIA

Neutropenia refers to the peripheral blood absolute neutrophil count (ANC) of $<1,500/\mu\text{L}$. Neutropenia is considered mild when ANC is between 1,000 and 1,500/ μL , moderate when ANC is between 500 and 1,000/ μL , and severe when ANC is $<500/\mu\text{L}$. Severe neutropenia is also referred to as *agranulocytosis* (Figure 22.10). Three major mechanisms are involved in the development of neutropenia: (1) reduced production or ineffective granulopoiesis,

TABLE 22.1 Major causes of neutropenia.

<i>Infections-associated</i>
<i>Drug-induced</i>
<i>Immune-associated</i> Isoimmune neonatal neutropenia Chronic autoimmune neutropenia Transfusion neutropenia Transfusion reactions
<i>Associated with hematologic disorders</i> Myelodysplastic syndromes Aplastic anemia Leukemia Bone marrow replacement by fibrosis or metastasis Hypersplenism
<i>Congenital neutropenia</i> Kostmann syndrome Congenital cyclic neutropenia
<i>Associated with other congenital anomalies</i> Schwachman–Diamond syndrome Cartilage-hair hypoplasia
<i>Associated with functional abnormalities</i> Chediak–Higashi syndrome Myelokathexis
<i>Other types of neutropenia</i> Acquired cyclic neutropenia Neutropenia due to nutritional deficiencies Neutropenia associated with endocrine disorders Lazy leukocyte syndrome Chronic idiopathic neutropenia

(2) increased destruction or utilization of neutrophils, and (3) a shift from the circulating to the marginal pool [48–50]. Neutropenia is either acquired or congenital [48]. Infections and drugs are the most common etiologic factors in acquired neutropenia. Other contributing causes include primary immune deficiencies, bone marrow disorders, and congenital factors (Table 22.1).

Post-Infectious Neutropenia

Post-infectious neutropenia is the most common type of neutropenia caused by relocation and accumulation of granulocytes from circulation into the infected sites and/or destruction by circulating antibodies [48, 49]. Severe forms of neutropenia have been reported in hepatitis B virus and EBV and HIV infections [48].

Drug-Induced Neutropenia

Drug-induced neutropenia is the second most common type of neutropenia caused by two possible mechanisms: (1) drug-induced cytotoxicity and (2) immune-mediated. The drug-induced cytotoxicity affects protein synthesis or cell

replication in the granulocytic precursors and is usually dose-dependent. Drugs or their metabolites may be the source of haptens or antigens, causing antibody production and drug–antibody interactions with neutrophils and their destruction [51]. Antithyroid drugs, clozapine, and sulfasalazine are among the drugs that could cause severe neutropenia [48]. A list of the drugs which may induce neutropenia is presented in Table 22.2. Drug-induced neutropenia is often associated with severe infectious complications and has a mortality rate of about 10% [52, 53].

Neutropenia Associated with Primary Immune Disorders

Neutropenia associated with primary immune disorders is caused by antineutrophil antibodies which are either autoimmune or alloimmune [51]. The mechanism of neutrophil destruction is either through complement-mediated neutrophil lysis or by splenic sequestration of opsonized neutrophils [49, 54]. The immune-associated neutropenia includes isoimmune neonatal neutropenia secondary to transplacental IgG antineutrophil antibodies, chronic autoimmune neutropenia primarily occurring in children younger than 4 years, transfusion reactions, complement activation, pure white cell aplasia due to antibody-mediated GM-CFU inhibitory activity, or antibodies to G-CSF [49].

Non-immune Chronic Idiopathic Neutropenia

Non-immune chronic idiopathic neutropenia is an acquired syndrome with no underlying autoimmune disease, nutritional deficiency, drug-association, or clonal bone marrow disorders [55]. The clinical course is usually benign and neutropenia is an incidental laboratory finding, with no history of infection or other symptoms [51, 56].

Neutropenia Associated with Bone Marrow Disorders

Neutropenia associated with bone marrow disorders include aplastic anemia, myelodysplastic syndromes, leukemias, and post-chemotherapy neutropenia.

Congenital Neutropenias

Congenital neutropenias consist of two major forms: cyclic neutropenia and severe congenital neutropenia.

Cyclic neutropenia is characterized by oscillation of peripheral neutrophil counts, from <500/μL to near normal range, with approximately 3 week intervals. The congenital form is autosomal dominant and is associated with mutations in the neutrophil elastase gene, *ELA2* [57]. The acquired forms of cyclin neutropenia have been observed in various conditions, such as chronic myelogenous leukemia, large granular lymphocytosis, and hypereosinophilic syndromes [48].

Severe congenital neutropenia refers to various congenital syndromes including infantile agranulocytosis

TABLE 22.2 Major drugs with potential risk of severe neutropenia.

<i>Antidepressant and psychotropic drugs</i>
Clozapine
Phenothiazines
Meprobamate
Tricyclic and tetracyclic antidepressants
<i>Antithyroid drugs</i>
Methimazole
Carbimazole
Propylthiouracil
<i>Antiinflammatory drugs</i>
Sulfasalazine
Gold salts
Penicillamine
Phenylbutazone
Dipyron
Phenacetin
<i>Antihistamines: H₂-receptor blockers</i>
Cimetidine
Ranitidine
<i>Antibacterial and antifungal drugs</i>
Chloramphenicol
Sulfonamides
Vancomycin
Cephalosporin
Amphotricin B
Flucytosine
Sulfamethoxazole
<i>Antimalarial</i>
Amodiaquine
Chloroquine
Quinine
<i>Cardiovascular drugs</i>
Propranolol
Dipyridamole
Digoxin
Antiarrhythmic drugs
ACE inhibitors
<i>Anticonvulsants</i>
Carbamazepine
Phenytoin
Ethosuximide
Valproate
<i>Diuretics</i>
Thiazides
Acetazolamide
Furosemide
Spironolactone
<i>Others</i>
Aminoglutethimide
Chlorpropamide
Tolbutamide
Isotretinoin
Dapsone

(Kostmann syndrome), Shwachman–Diamond–Osaki syndrome, myelokathexis, CHS, and congenital dysgranulopoietic neutropenia [48, 49].

Kostmann syndrome is an autosomal recessive agranulocytosis of infancy characterized by frequent, severe infections, mostly due to staphylococci and streptococci [58]. Bone marrow often reveals myeloid hypoplasia and maturation arrest at the promyelocyte stage [59].

Shwachman–Diamond–Osaki syndrome represents a triad of neutropenia, pancreatic insufficiency, and metaphyseal dysplasia [50]. Patients are usually under 10 years of age with a history of recurrent infection and steatorrhea. Neutropenia appears to be secondary to increased apoptosis in the bone marrow [60].

Myelokathexis is an extremely rare form of chronic, childhood neutropenia with recurrent infections [61–65]. The bone marrow is often hypercellular and shows dysmyelopoietic features [63]. The dysplastic changes include nuclear hypersegmentation, cytoplasmic vacuolization, and hypogranularity [61]. One possible mechanism for neutropenia is defective release and prolonged retention of neutrophils in the bone marrow [61, 63]. Myelokathexis has been reported in association with accelerated apoptosis and defective expression of bcl-x in granulocytic precursors. It is considered a part of WHIM (warts, hypogammaglobulinemia, infections, myelokathexis) syndrome [65–67].

Chediak–Higashi syndrome is described in Chapter 21.

Congenital dysgranulopoietic neutropenia is a rare autosomal recessive disorder characterized by repeated severe infections and dysmyelopoietic features in the bone marrow, including defects in the synthesis of primary and specific granules and premature cell lysis [68, 69].

Neutropenia has also been associated with lazy leukocyte syndrome, bone marrow stem cell disorders (such as aplastic anemia, leukemias, and myelodysplastic syndrome, in cobalamin deficiency), and hereditary disorders, such as dyskeratosis congenital, reticular dysgenesis, and glycogen storage disease type 1 [48, 49]. Lazy leukocyte syndrome is an extremely rare condition characterized by severe neutropenia, defective neutrophil chemotaxis, and impaired random mobility of granulocytes [70, 71].

NEUTROPHILIA

Neutrophilia refers to an increase in the absolute number of neutrophils in the peripheral blood (absolute neutrophil count $>7,700/\mu\text{L}$ in adults). Since neutrophils account for the majority of the circulating leukocytes (about 60%), in most instances, white blood cell (WBC) counts of over $11,000/\mu\text{L}$ represent neutrophilia [72]. There are two major causes for neutrophilia: (1) a reactive response to ongoing processes, such as infection, inflammation, smoking, stress, medication, or malignancy and (2) due to primary abnormalities in the regulation of bone marrow neutrophil production (Table 22.3). Leukemoid reaction is referred to leukocytosis in excess of $50,000/\mu\text{L}$ with a left shift, caused by conditions other than a leukemic process [72].

TABLE 22.3 Major causes of neutrophilia.

<i>Reactive</i>
Acute infections
Chronic inflammations
Cigarette smoking
Exercise
Stress
Drugs
Bone marrow stimulation
Non-hematopoietic malignancies
Heatstroke
Others
<i>Primary</i>
Hereditary neutrophilia
Down syndrome
Muckle–Wells syndrome
Leukocyte adhesion deficiency
Chronic myeloproliferative disorders
Others

Reactive Neutrophilia

Acute infections commonly cause various degrees of neutrophilia due to the release of segmented neutrophils and bands from the bone marrow and marginating pool (Figure 22.11). Acute bacterial infections, such as pneumococcal, staphylococcal, or leptospiral infections, are the most frequent causes of infection-induced neutrophilia. Certain viral infections, such as herpes complex, varicella, and EBV infections, may also cause neutrophilia. Reactive neutrophilia is often associated with the presence of toxic granulation, Dohle bodies, cytoplasmic vacuoles, and elevated levels of leukocyte (neutrophil) alkaline phosphatase (LAP) (Figure 22.11c).

Chronic inflammations, such as rheumatoid arthritis, Kawasaki disease, and inflammatory bowel disorders are often associated with neutrophilia. Neutrophilia in these conditions are in part due to the release of cytokines, such as TNF- α , G-CSF, GM-CSF, IL-6, and IL-8 [72–74].

Cigarette smoking is usually associated with elevated leukocyte count and neutrophilia (up to 25%), which may last as long as 5 years after quitting smoking [74–77]. The exact mechanism of smoking-induced leukocytosis is not known.

Stress-associated neutrophilia appears to be related to the redistribution of neutrophils from the marginating pool into the circulating pool, probably due to the reduced neutrophil adhesion by the release of epinephrine [78]. Post-operative neutrophilia and leukocytosis during acute myocardial infarction are considered stress-related [79, 80].

Exercise-induced neutrophilia is probably due to a combination of increased plasma epinephrine levels and a change in the cardiac output, leading to the redistribution of neutrophils from the marginating pool into the circulating pool [81, 82]. Delayed leukocytosis in exercise is probably due to the release of leukocytes from the bone marrow [83].

Medications such as beta agonists (e.g. epinephrine), glucocorticosteroids, lithium, recombinant colony stimulating factors, and all-*trans* retinoic acid (ATRA) are associated with neutrophilia (Figure 22.12) [84–86].

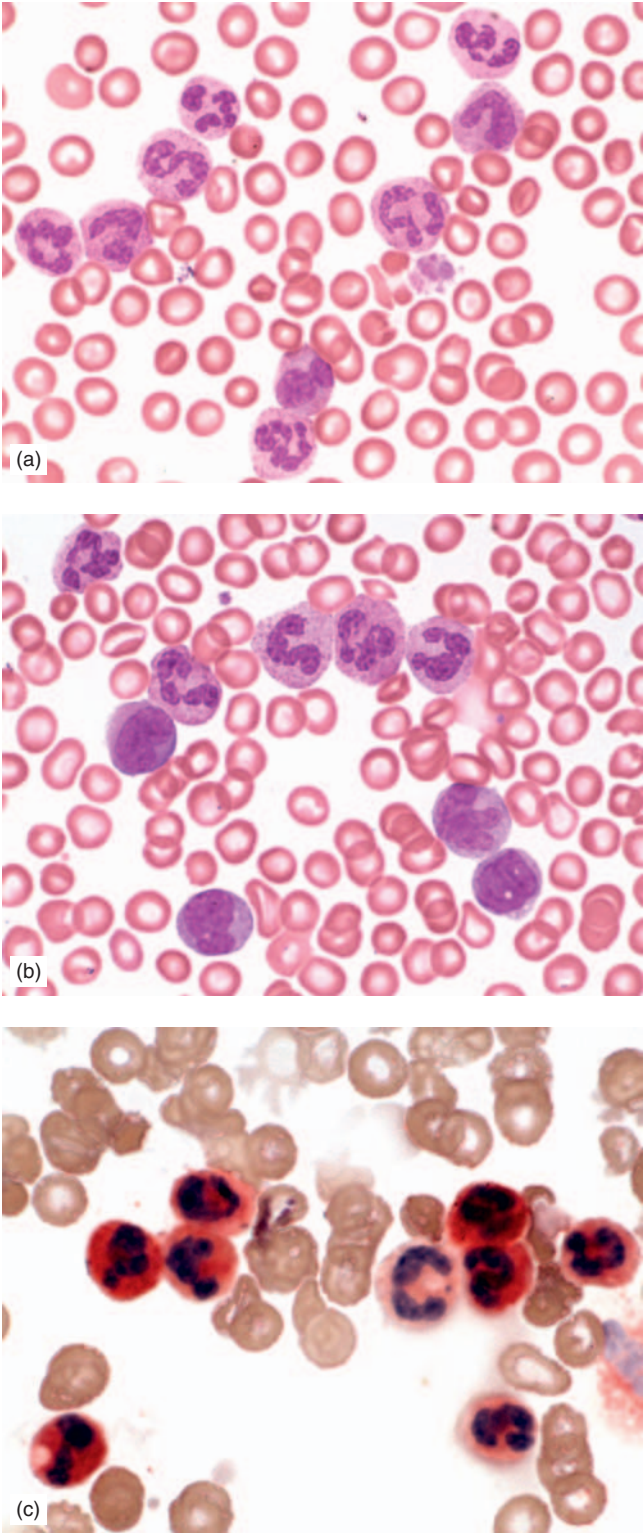


FIGURE 22.11 Leukemoid reaction. Blood smears demonstrate numerous neutrophils and bands (a), neutrophils and monocytes (b), and alkaline phosphatase-positive neutrophils (c).

Other causes of neutrophilia include bone marrow stimulation (such as hemolytic anemia or immune thrombocytopenia), non-hematologic malignancies, heatstroke, and post-splenectomy.

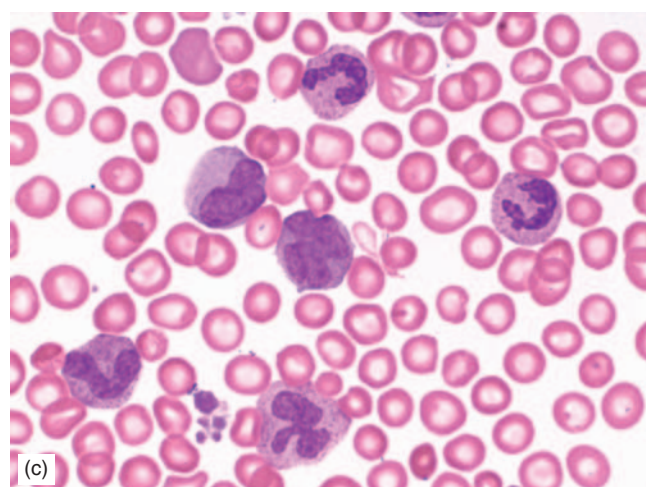
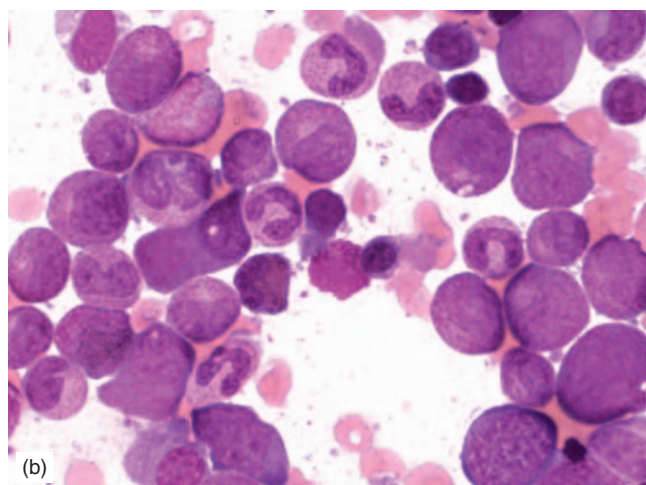
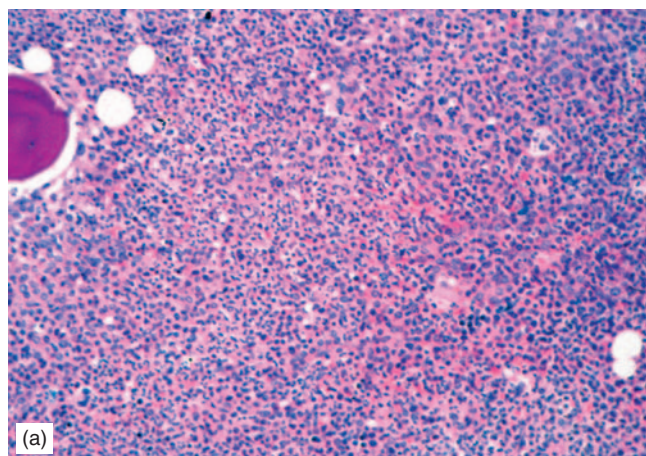


FIGURE 22.12 Effect of G-CSF therapy. Bone marrow biopsy section (a) and smear (b) show myeloid preponderance and left shift. Blood smear (c) reveals leukocytosis with the presence of neutrophils and monocytes.

Primary Neutrophilia

Primary neutrophilia is due to primary abnormalities in the regulation of bone marrow neutrophil production, such as

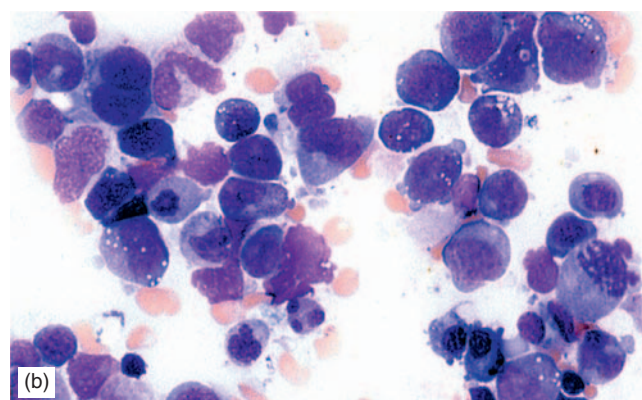
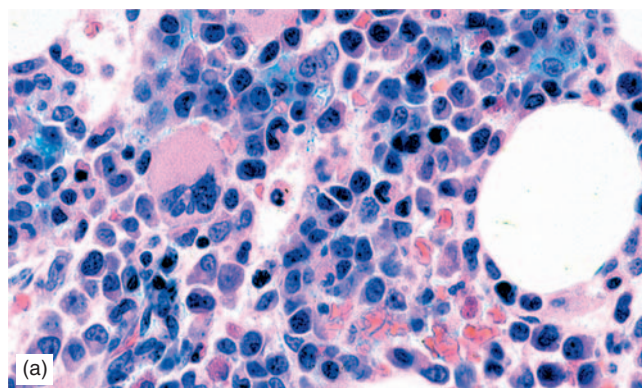


FIGURE 22.13 Transient myeloproliferative disorder in Down syndrome. Bone marrow biopsy section (a) and smear (b) show myeloid preponderance and left shift.

hereditary neutrophilia, chronic myeloproliferative disorders (see Chapter 9), myeloproliferative/myelodysplastic syndromes (see Chapter 10), transient myeloproliferative disorder in Down syndrome, and leukocyte adhesion deficiency. In this section, hereditary neutrophilia and transient myeloproliferative disorder in Down syndrome are briefly discussed.

Hereditary neutrophilia is a rare autosomal disorder characterized by chronic neutrophilia (ranging from 20,000 to over 100,000/ μ L), splenomegaly, elevated LAP, and widened dipole of the skull [87]. Neutrophil function is normal, but affected individuals may demonstrate bleeding complications due to platelet dysfunction [87].

Transient myeloproliferative disorder (TMD) in Down syndrome or transient abnormal myelopoiesis is a leukemoid reaction occasionally observed in some neonates with Down syndrome (trisomy of chromosome 21) [80–90]. The affected neonates are usually under 1 month old and demonstrate peripheral blood leukocytosis with a left shift and the presence of blast cells (Figures 22.13 and 22.14). Blast cells are predominantly of megakaryocytic and erythroid origin. Mutation of *GATA-1* gene encoding for erythroid/megakaryocytic transcription factor GATA-1 has been reported in Down syndrome patients with TMD [91]. TMD in Down syndrome usually disappears spontaneously after 4–6 weeks [91, 92]. A small proportion of patients may eventually develop acute myelogenous leukemia [93, 94].

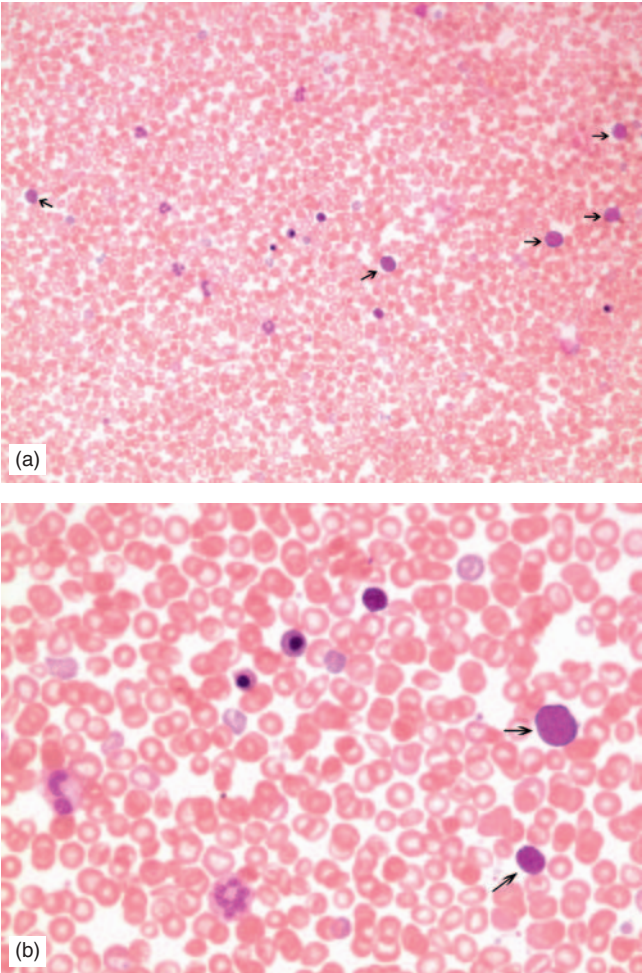


FIGURE 22.14 Transient myeloproliferative disorder in Down syndrome. Blood smear demonstrate several blast cells: (a) low power and (b) high power views.

Spurious Neutrophilia

Spurious neutrophilia is a falsely elevated peripheral blood neutrophil count resulting from various reasons. Formation of precipitated cryoglobulin particles may result in erroneous elevated WBC and/or platelet counts [95]. Also, platelet clumps may be counted as leukocytes by automated cell counters.

EOSINOPHILIA

Eosinophilia is defined as an absolute peripheral blood eosinophil count exceeding 600/ μ L [96]. It is observed in a wide variety of conditions including allergic and inflammatory processes, protozoan and metazoan infections, immunodeficiencies and autoimmune disorders, chronic myeloproliferative disorders, leukemias and lymphomas, and certain non-hematopoietic malignancies (Table 22.4). Idiopathic hypereosinophilic syndrome and chronic eosinophilic leukemia are classified under chronic myeloproliferative disorders and are discussed in Chapter 9.

TABLE 22.4 Conditions associated with eosinophilia.*

Protozoan infections
Pneumocystic, toxoplasmosis, amebiasis, malaria
Metazoan infections
Nematodes, trematodes, cestodes, arthropods
Allergic and autoimmune disorders
Hay fever, asthma, angioneurotic edema, urticaria, serum sickness, allergic vasculitis, pemphigus vulgaris, dermatitis herpetiformis, ulcerative colitis, regional enteritis
Hematopoietic disorders
Hodgkin and non-Hodgkin lymphomas, mycosis fungoides, acute myelogenous leukemia, chronic myeloproliferative disorders, plasma cell myeloma, Langerhans cell histiocytosis, familial hemophagocytic lymphohistiocytosis
Solid tumors
Carcinomas, brain tumors, melanoma
Others
Immunodeficiency syndromes, sarcoidosis, chronic renal disease, peritoneal dialysis, pleural effusion, radiotherapy, splenectomy

*Adapted from Ref. [3].

A hypereosinophilic syndrome has been reported in association with the use of tryptophan derivatives [97, 98]. It is characterized by peripheral blood eosinophilia and scleroderma-like features, including muscle tenderness, fatigue, edema, arthralgia, nephropathy, rash, cough, and dyspnea. Tryptophan derivatives have been used for the treatment of insomnia and depression.

Eosinophilia is reported in association with a variety of solid tumors such as bronchogenic carcinoma, medullary carcinoma of the thyroid gland, and transitional cell carcinoma of the bladder. A garden variety of hematopoietic malignancies, such as chronic myelogenous leukemia, acute lymphoblastic leukemia, acute myelogenous leukemia, and Hodgkin and non-Hodgkin lymphomas, may demonstrate eosinophilia in the involved tissues and peripheral blood. A subtype of acute myelomonocytic leukemia is associated with atypical eosinophilia and chromosomal aberrations involving 16q22 (see Chapter 10). A subtype of precursor B-cell acute lymphoblastic leukemia with t(5;14) has been associated with eosinophilia [99, 100]. Similarly, eosinophilia has been reported in precursor T-cell lymphoblastic leukemias with t(8;13) [101].

BASOPHILIA

Basophilia is defined as an absolute peripheral blood basophil count exceeding 200/ μ L [72]. It may occur in a wide variety of infections or inflammatory and autoimmune conditions, such as tuberculosis, chickenpox, smallpox, influenza, ulcerative colitis, and rheumatoid arthritis [72, 102, 103]. Other conditions associated with basophilia

include irradiation, iron deficiency, hypothyroidism, diabetes mellitus, chronic myeloproliferative disorders, and myelodysplastic syndromes [104]. Certain subtypes of acute myelogenous leukemia, such as acute promyelocytic leukemia and acute myeloid leukemias with t(6;9), t(3;6), and aberrations of chromosome 16 may demonstrate bone marrow basophilia [105].

MASTOCYTOSIS

Mastocytosis is discussed in Chapter 20.

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Disorder of Red Blood Cells: Anemias

Faramarz Naeim

Anemia, a decline in blood hemoglobin (Hb) level, is caused by three major mechanisms: (1) blood loss, (2) inefficient erythropoiesis, and (3) increased red blood cell (RBC) destruction. These mechanisms are often associated with certain morphologic features reflecting the size (normocytic, microcytic, or macrocytic) or the Hb content (normochromic, hypochromic, or hyperchromic) of the erythrocytes.

Four RBC indices are measured by automated hematology instruments in clinical laboratories. These indices correlate with the size, hemoglobin content, and degree of anisocytosis in the RBCs. They include (Table 23.1):

1. Mean corpuscular volume (MCV) calculated as: $\text{HCT (\%)} \times 10 / \text{RBC count (million}/\mu\text{L)}$. MCV indicates average RBC volume.
2. Mean corpuscular hemoglobin (MCH) calculated as: $\text{Hb (g/dL)} \times 10 / \text{RBC count (million}/\mu\text{L)}$. MCH indicates average amount of Hb per RBC.

3. Mean corpuscular hemoglobin concentration (MCHC) calculated as: $\text{Hb (g/dL)} \times 100 / \text{HCT (\%)}$. MCHC indicates the average concentration of Hb per RBC.

4. Red cell distribution width (RDW) indicates the degree of anisocytosis.

Biochemical analyses such as measurement of serum iron, iron-binding capacity, ferritin, folate, and vitamin B₁₂ levels, RBC enzyme assays, and Hb electrophoresis provide valuable information regarding the cause of anemia. Molecular genetic studies add additional dimensions to the understanding, classification, and treatment of certain anemias, particularly the hereditary variants.

Routine examination of the peripheral blood plays a key role in the diagnosis and classification of anemias. It provides basic information regarding RBC counts, morphology and indices, Hb and hematocrit (HCT, percentage of

TABLE 23.1 Red blood cell (RBC) values and related parameters in healthy adults.*

Parameters	Men	Women	Both
RBC count, million/ μL	$5.2 \pm 0.7^{**}$	4.6 ± 0.5	
Hemoglobin (Hb), g/dL	15.7 ± 1.7	13.8 ± 1.5	
Hematocrit, %	46.0 ± 4.0	40.0 ± 4.0	
Reticulocytes, %	1.6 ± 0.5	1.4 ± 0.5	
Mean corpuscular volume, fL (MCV)			88.0 ± 8.0
Mean cell hemoglobin, pg/RBC (MCH)			30.4 ± 2.8
Mean cell Hb concentration, g/dL of RBC (MCHC)			34.4 ± 1.1
Red cell volume distribution width (RDW), %			13.1 ± 1.4

*Adapted from Butler E, Lichtman MA, Collier BS, et al. (2001). *Williams' Hematology*, 6th ed., McGraw-Hill, New York.

**Values = mean + standard deviation.

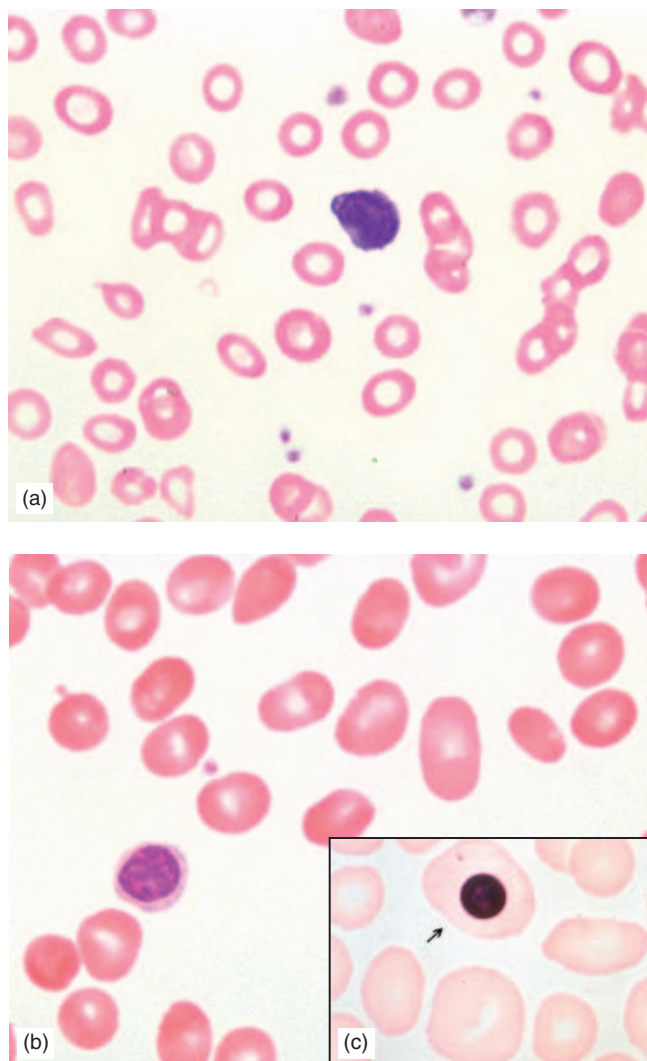


FIGURE 23.1 (a) Blood smear demonstrating microcytic hypochromic erythrocytes in a patient with iron deficiency anemia. (b and c) Macro-ovalocytes in a patient with megaloblastic anemia. A late stage nucleated red cell with abundant cytoplasm is present (c, arrow).

the packed RBC volume in blood) levels (Table 23.1). The WHO criteria for anemia are <13 and $<12\text{g/dL}$ of Hb for men and women, respectively [1, 2], or more than two standard deviations (SD) below the mean of normal range in an age- and sex-matched representative population [3]. In general, the range of Hb, HCT, and RBC counts are higher in smokers and in people who live in an air-polluted environment or at high altitudes, and are lower in women, Afro-Americans, and the elderly [3–7].

Morphologic variations are often associated with certain categories of anemias. For example, microcytic, hypochromic RBCs are seen in iron deficiency anemia (IDA), thalassemia, and lead poisoning (Figure 23.1a); macrocytic RBCs are observed in folate or vitamin B₁₂ deficiencies, liver disease, hypothyroidism, and newborns (Figure 23.1b and c); target cells are often associated with thalassemia and IDA (Figure 23.2a); stomatocytes are seen in hereditary conditions, liver diseases, and electrolyte imbalances (Figure

23.2b); burr cells (echinocytes) are found in uremia, pyruvate kinase (PK) deficiency, and acute blood loss (Figure 23.3a); acanthocytes may be present in a β -lipoproteinemia, liver diseases, and anorexia nervosa (Figure 23.3b); and fragmented RBCs (schistocytes) are seen in microangiopathic hemolytic anemias such as disseminated intravascular coagulopathies, thrombotic thrombocytopenic purpura, and severe burns (Figure 23.4a). Teardrop-shaped erythrocytes (dacrocytes) are seen in bone marrow fibrosis, thalassemia syndromes, and hemolytic anemias (Figure 23.4b). Reticulocytes (polychromatophilic RBCs) account for about 0.5–1.5% of the RBCs and are primarily increased in conditions associated with elevated production of bone marrow erythropoiesis, such as hemolytic anemias (Figure 23.5).

Erythrocytes may demonstrate a variety of cytoplasmic inclusions. For example, basophilic stippling is seen in lead poisoning, impaired Hb synthesis, alcoholism, and megaloblastic anemias (Figure 23.6a), and iron particles (Pappenheimer bodies) are noted in myelodysplastic syndrome (MDS), congenital dyserythropoietic anemia, and post-splenectomy (Figure 23.6b). Erythrocytes may contain remnants of DNA (Howell-Jolly bodies), such as in megaloblastic anemia or after splenectomy. Cabot rings are inclusions observed in pernicious anemia or lead poisoning (Figure 23.7). Heinz bodies (precipitated abnormal Hb structures) and Hb H inclusions are visible by supravital stains (Figure 23.8). RBCs may also contain a variety of microorganisms (Figure 23.9).

Although peripheral blood is the most informative sample in the diagnosis and classification of anemias, bone marrow examination, in certain conditions, provides additional valuable information such as bone marrow cellularity, myeloid:erythroid (M:E) ratio, estimation of stored iron and presence or lack of bone marrow replacement by fibrosis, inflammatory processes, or primary or secondary malignancies. In general, anemia caused by blood loss or RBC destruction is associated with bone marrow erythroid hyperplasia and reticulocytosis, whereas anemia due to ineffective erythropoiesis is characterized by reticulocytopenia and a bone marrow which may be hypo-, normo-, or hypercellular.

In this chapter, most anemias, especially those associated with significant bone marrow changes, are discussed. Anemias secondary to the pluripotent hematopoietic stem cell disorders such as aplastic anemia, chronic myeloproliferative disorders, and myelodysplastic syndromes are discussed in previous chapters.

PURE RED CELL APLASIA

Pure red cell aplasia (PRCA) is a rare condition characterized by severe anemia, lack of reticulocytes, and marked reduction or virtual absence of erythroid precursors in the bone marrow.

Etiology and Pathogenesis

The primary defect in PRCA appears to be the inability of the committed erythroid progenitor cells, BFU-E and

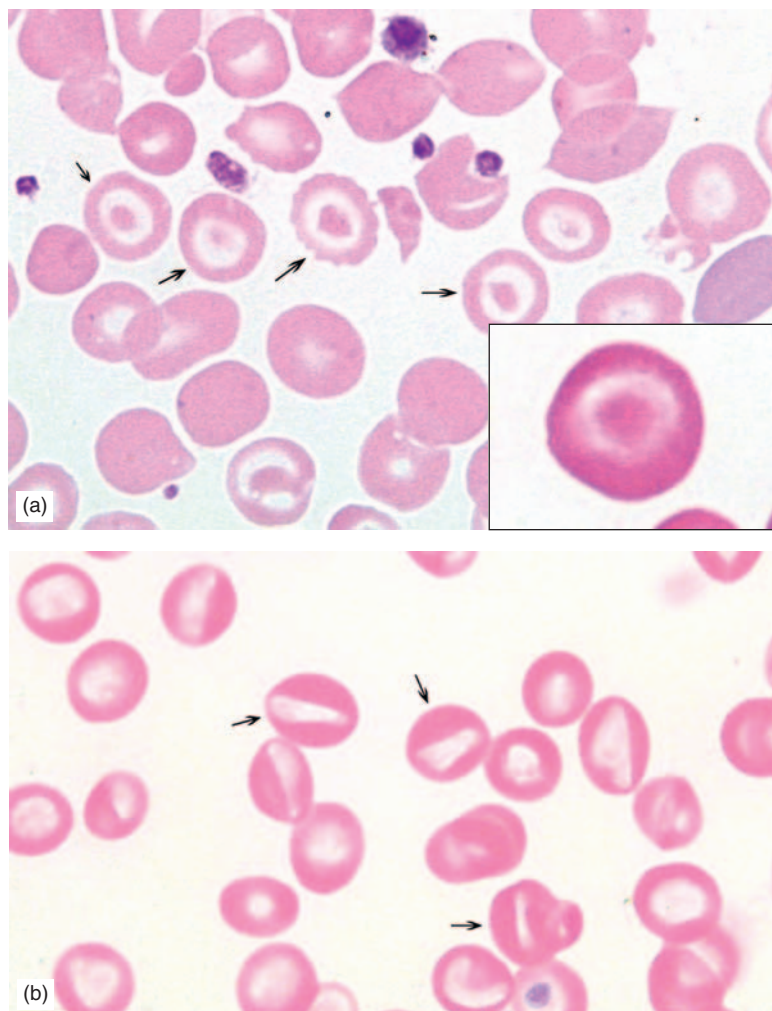


FIGURE 23.2 Blood smears demonstrating target cells (a, arrows and inset) and stomatocytes (b).

CFU-E, to differentiate into pronormoblasts [8]. There are two forms of PRCA: congenital and acquired.

The congenital form or the Diamond–Blackfan anemia apparently represents a heterogeneous group and has been observed in both autosomal recessive and dominant forms [9–11]. A significant proportion of these patients show mutations on chromosomes 19q or 8p [10, 12]. The 19q mutation involves *RPS19* gene, encoding the ribosomal protein S19, suggesting that a defect in ribosome biogenesis plays a role in the pathogenesis of Diamond–Blackfan anemia [13, 14].

Acquired PRCA may be drug-induced or associated with a wide variety of clinical conditions such as viral infections, autoimmune disorders, and lymphoid malignancies (Table 23.2) [3, 8, 14, 15]. Acquired PRCA may also precede myelodysplastic syndrome [14, 15].

The B19 parvovirus-induced PRCA can occur in various conditions such as sickle cell (SC) anemia, post-organ transplantation, congenital immunodeficiency syndromes, and lymphoproliferative disorders [16–21]. The erythroid progenitor cells are targeted by B19 parvovirus via the red cell receptor globoside (blood group P antigens) [19].

The majority of immunologically mediated PRCAs are caused by antibodies which either inhibit erythropoiesis and Hb synthesis or complement-binding and have direct cytotoxic effects on erythroblasts [22, 23].

Pathology and Laboratory Findings

Patients usually have a profound anemia that is often macrocytic. White blood cell (WBC) and platelet counts are unaffected. Bone marrow specimens of patients with PRCA show variable cellularity with markedly elevated M:E ratio due to severe erythroid depletion and rare late erythroid progenitor cells (Figure 23.10). The erythroid cells may appear dysplastic. Parvovirus-associated aplasia is often characterized by prominent megaloblastic changes and the presence of giant rubriblasts (gigantoblasts) (Figures 23.11–23.13). These giant cells are characterized by dark basophilic cytoplasm and large vesicular nuclei with fine chromatin and prominent nucleoli [24]. They may demonstrate viral inclusions. The granulocytic and megakaryocytic lines are unremarkable and show progressive maturation. No significant changes are noted in the lymphocytic and plasmacytic population, except when the underlying cause is a lymphoproliferative process.

Anemia is severe and macrocytic [3, 16, 18]. The majority of the patients show an elevated red cell adenosine deaminase activity [25] and may also demonstrate an increased proportion of fetal Hb for age and an increased expression of the I antigen [26].

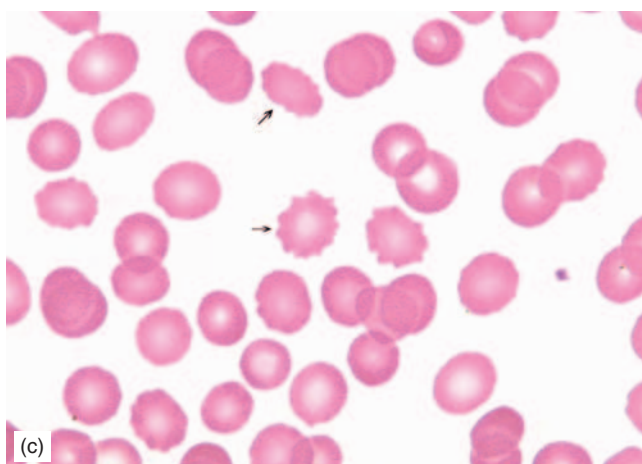
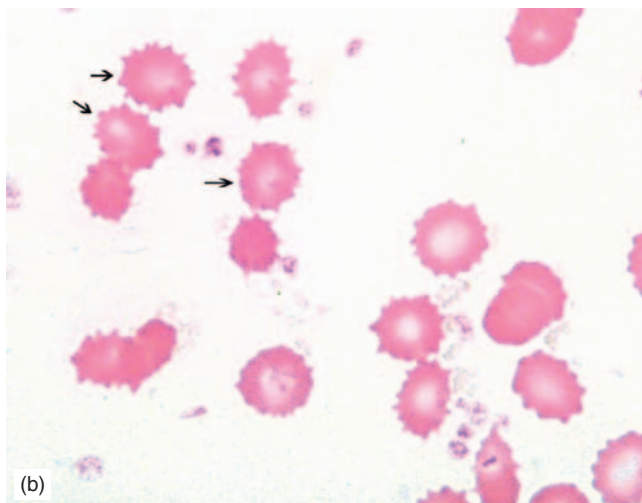
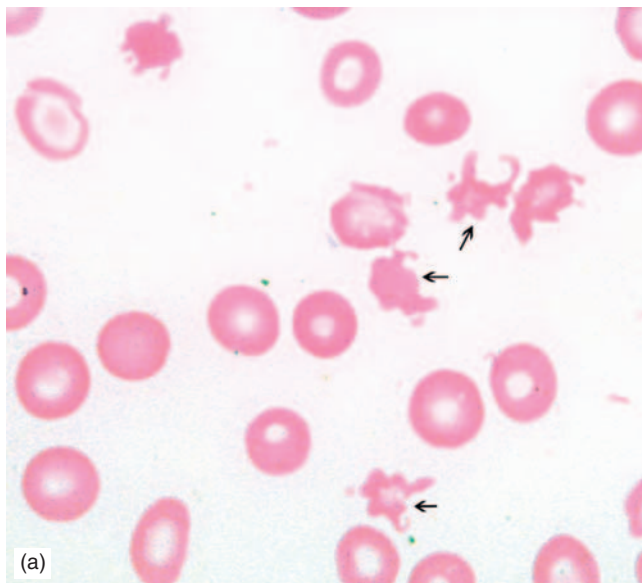


FIGURE 23.3 Blood smears demonstrate acanthocytes (a) with irregularly spaced, thorn-like projections, echinocytes (b) with evenly spaced pointed projections, and crenated red cells (c) with blunt projections evenly distributed over the surface. Crenated red cells are usually caused by faulty drying of the blood smear, change in pH, or excess EDTA (shrinkage of the erythrocytes).

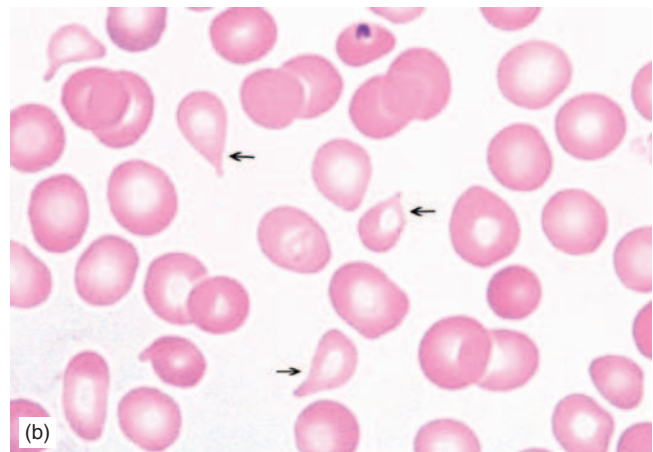
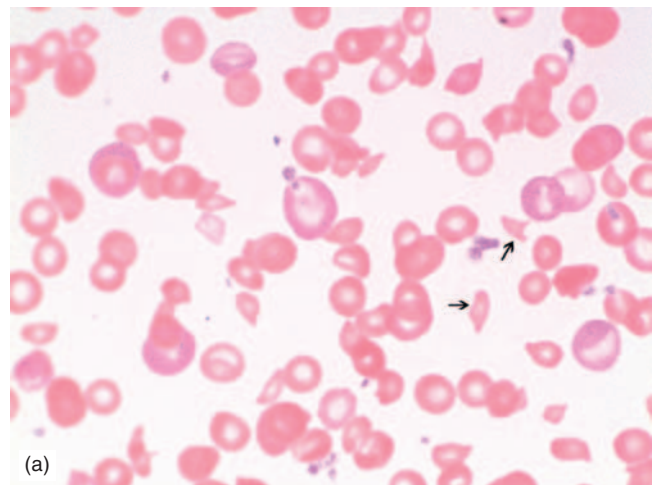


FIGURE 23.4 Blood smears demonstrate fragmented red cells (schistocytes) (a) and teardrop red cells (dacrocytes) (b, arrows).

Clinical Aspects

Diamond–Blackfan anemia is a disease of early infancy with over 90% of cases diagnosed within the first year of life [9, 16–18, 26]. Males and females are equally affected. The disease is sporadic in about 35% of the cases, but autosomal recessive and dominant forms have been reported in several families [16]. Approximately one-third of the patients with Diamond–Blackfan anemia demonstrate physical abnormalities such as short stature, microcephali, cleft palate, atrial or ventricular septal defect, and/or urogenital abnormalities [27]. Spontaneous remissions have been reported in up to 25% of the patients [28]. Therapeutic approaches include corticosteroid and metoclopramide therapy, blood transfusion, and bone marrow transplantation [29, 30].

The *transient erythroblastoma of childhood* is a temporary acquired anemia with reticulocytopenia and bone marrow erythroblastopenia usually observed in children <1 year old [28, 31]. It is mostly idiopathic or in some cases autoimmune related.

Major underlying causes of non-congenital forms of PRCA are large granular lymphocytic leukemia, thymoma, hypoplastic MDS, parvovirus B19, HIV, drugs, autoimmune

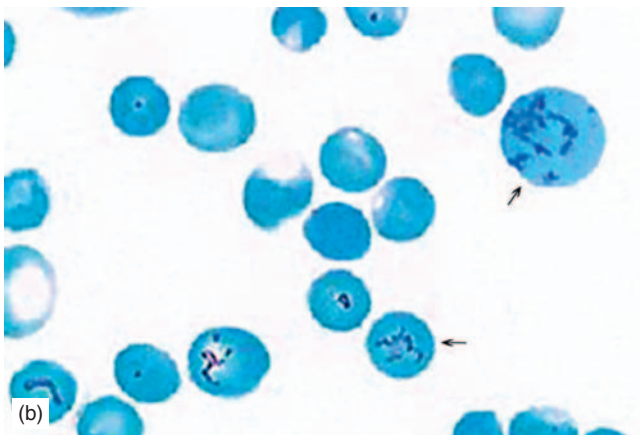
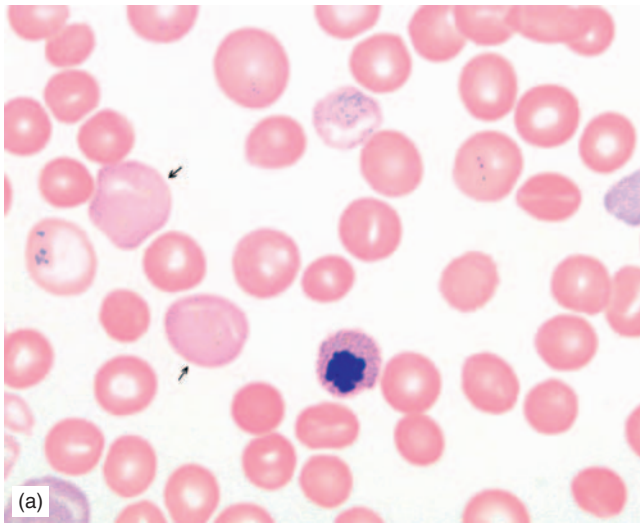


FIGURE 23.5 Polychromatophilic red cells (reticulocytes) (a, arrows) contain ribosomes, which are demonstrated by supravital stains (b, arrows).

disorders, and idiopathic (Table 23.2) [18–20, 32–34]. Therapeutic approaches include treatment of the underlying disorder and supportive therapy.

Differential Diagnosis

The major distinguishing features of Diamond–Blackfan anemia are family history and the manifestation of the disease in early infancy. Patients may also show mutations on chromosomes 19q or 8p. Anemia secondary to acquired PRCA is often transient and is mostly seen in individuals older than 1 year.

CONGENITAL DYSERYTHROPOIETIC ANEMIAS

Congenital dyserythropoietic anemias (CDAs) are a group of rare congenital anemias characterized by ineffective erythropoiesis and dysplastic changes in erythroid precursors [35–38]. Originally, there were three well-established CDA types, I, II, and III (Table 23.3), but several new

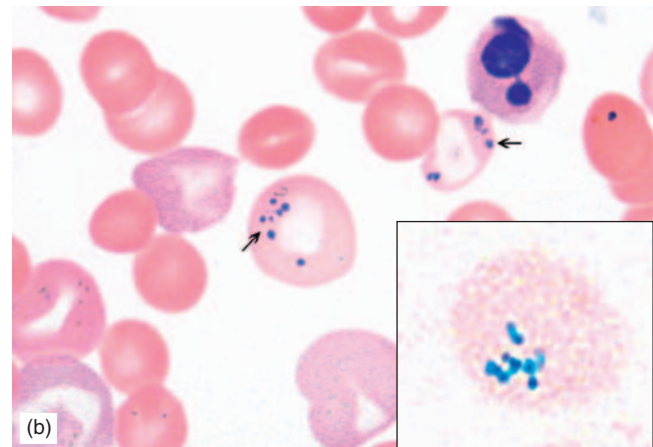
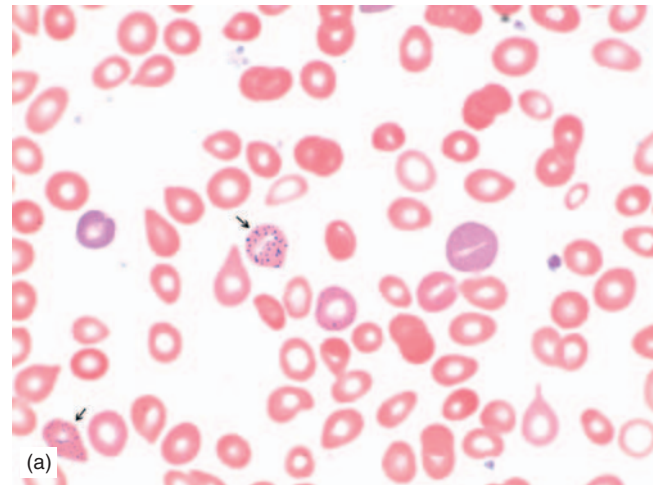


FIGURE 23.6 Blood smear showing basophilic stippling (a, arrows), polychromatophilic red cells, and teardrops. Dark, angular bodies of various sizes on Wright-stained smear (b) represent Pappenheimer bodies (iron particles). The inset is iron stain.

variants such as types IV, V, VI, and VII have been recognized in recent years [36, 37].

Congenital Dyserythropoietic Anemia Type I

Congenital dyserythropoietic anemia type I is a rare autosomal recessive disorder manifested in various ages, ranging from infancy to adulthood [39, 40]. It is characterized by variable degrees of anemia with mild to distinct macrocytosis, moderate hyperbilirubinemia, iron overload, and often splenomegaly [41, 42]. Congenital malformations such as presence of sixth toe, ventricular septal defect, short stature, and hip dysplasia have been reported in up to one-third of the cases [43]. The majority of the patients demonstrate mutations of *CDAN1* gene mapped to chromosome 15q15.1–15.3 [44–46].

The bone marrow is hypercellular with marked erythroid hyperplasia and dysplasia. Dysplastic changes are mostly confined to the middle and late stages of erythroid maturation and include nuclear irregularity, double segmented nuclei, and binucleation with nuclei of different sizes, textures, and stainability (Figures 23.14 and 23.15). Pairs of

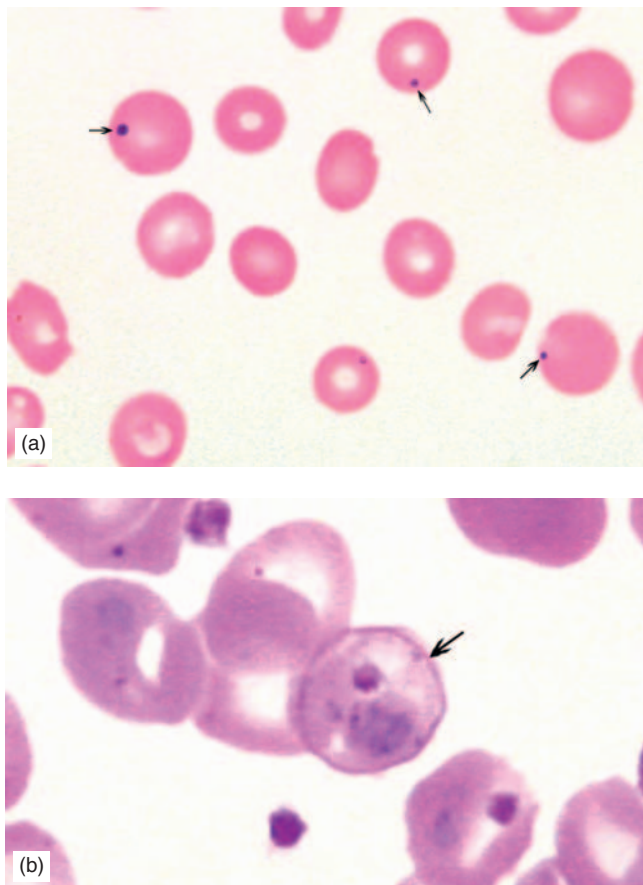


FIGURE 23.7 Blood smears demonstrate Howell-Jolly bodies (a, arrows) and a Cabot ring (b, arrow). Courtesy of Diana Tanaka-Mukai, Clinical Laboratories, UCLA Medical Center.

normoblasts attached by a chromatin bridge may be present. There are also mild to moderate megaloblastic changes. Iron stores are increased [37, 41, 45]. Electron microscopy reveals widening of the nuclear membrane pores with vacuolization and disintegration of nuclear chromatin (“Swiss cheese” appearance [47, 48]).

The peripheral blood shows anemia with Hb levels ranging from 6.5 to 13 g/dL. Anisocytosis, poikilocytosis, and macrocytosis are common features with a normal to slightly elevated reticulocyte count (1–5%) [45]. Basophilic stippling, Howell-Jolly bodies, and Cabot rings may be present. Some cases may show elevated Hb A₂ levels and/or unbalanced globin chain synthesis with increased $\alpha:\beta$ ratio [37]. The serum levels of haptoglobin are low, bilirubin is high, and iron is normal to elevated [37, 41, 45]. Patients with CDA type I show a significant positive response to recombinant interferon- α_2 treatments [44].

Congenital Dyserythropoietic Anemia Type II

Congenital dyserythropoietic anemia type II is an autosomal recessive disorder also known as *hereditary erythroblastic multinuclearity with a positive acidified serum lysis test*

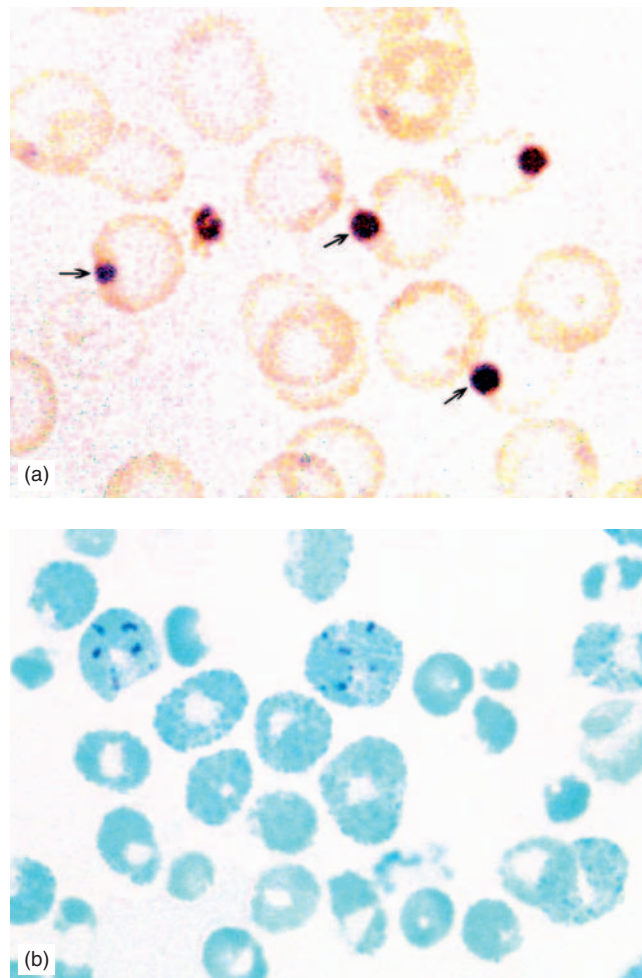


FIGURE 23.8 Heinz bodies are unstable Hb precipitates attached to red cell membrane visible by supravital stains (a, arrows). Hemoglobin H represented as small evenly dispersed deposits (like golf ball), visible by supravital stains (b). Courtesy of Diana Tanaka-Mukai, Clinical Laboratories, UCLA Medical Center.

(HEMPAS) (Table 23.3). CDA type II is the most common type of CDA with >300 cases reported. The extent of anemia varies from mild to severe. Hyperbilirubinemia and consequently gallstone formation are frequent findings and over two-thirds of the patients show splenomegaly. There is a progressive elevation of serum ferritin levels with about 20% chance of development of hepatic cirrhosis [49, 50]. Dysmorphic features are less common than type I [49]. Linkage studies have localized a gene (*CDAN2*) in the region of chromosome 20q11.2 [51].

The bone marrow is hypercellular and shows erythroid hyperplasia with dysplastic changes in middle to late stage normoblasts. A significant proportion of these cells (10–30%) show bi- or multilobated nuclei and many are binucleated. Overdestruction of erythroid precursors and increased cell debris often lead to the accumulation of macrophages and the presence of pseudo-Gaucher cells or sea-blue histiocytes. Electron microscopy reveals an excess of smooth endoplasmic reticulum parallel to the cell membrane of the normoblasts (“double membrane” appearance) [52].

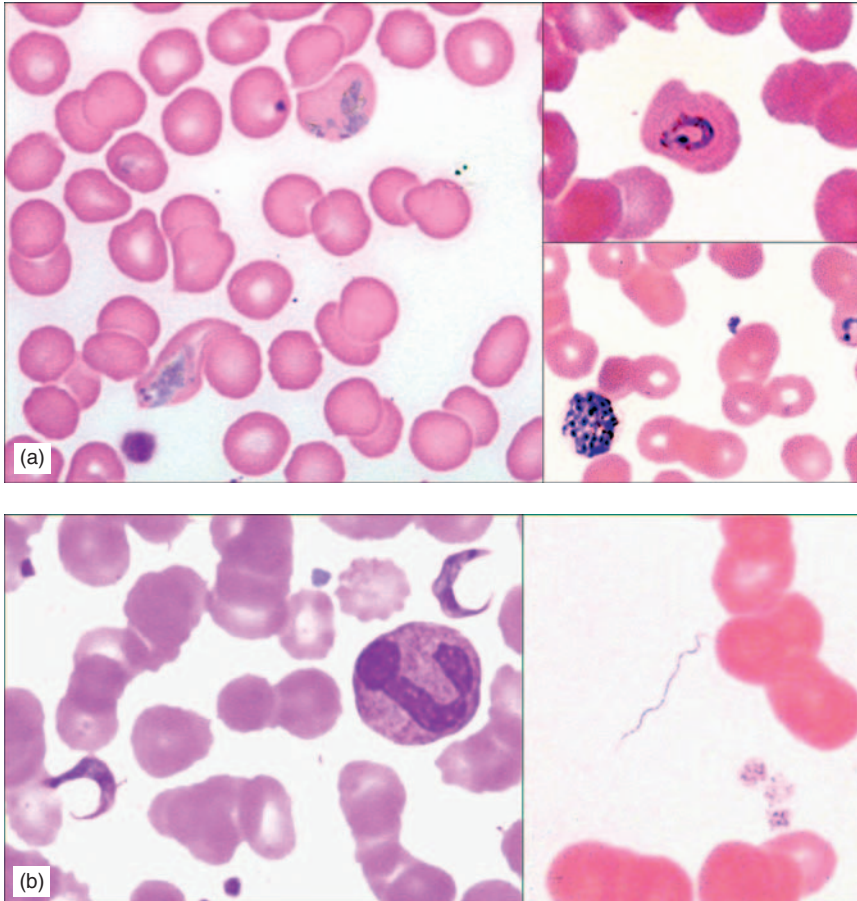


FIGURE 23.9 (a) Blood smears demonstrate ring and schizont forms of malarial parasites in the erythrocytes and (b) blood smears showing *Trypanosoma brucei* (left) and *Borrelia recurrentis* (right). Courtesy of Diana Tanaka-Mukai, Clinical Laboratories, UCLA Medical Center.

TABLE 23.2 Conditions associated with acquired pure red cell aplasia.*

Lymphoproliferative disorders
Large granular lymphocyte-mediated
B-cell lymphoproliferative disorders
Myelodysplastic syndromes
Myeloproliferative disorders
Viral infections (parvovirus B19, viral hepatitis, HIV)
Drugs (phenytoin, chloramphenicol, zidovudine)
Immunologically mediated
Autoimmune hemolytic anemia
Rheumatoid arthritis
Systemic lupus erythematosus
ABO-incompatible stem cell transplantation
Antibodies against erythropoietin
Thymoma and other cancers
Pregnancy
Idiopathic

*Adapted from Ref. [16].

The peripheral blood smear shows varying degrees of anisopoikilocytosis, often with a normal MCV. Occasional basophilic stippling or teardrops may be present. The RBCs of most patients are lysed in the acidified serum lysis test (Ham test) by normal sera but not by the patient's own serum [43].

Band 3 and 4.5 glycoproteins are underglycosylated, although there may be overglycosylation of glycolipids [49, 53, 54]. Therapeutic approaches include supportive therapy, such as RBC transfusion, and splenectomy if severity of anemia compromises patients' performance [49].

Congenital Dyserythropoietic Anemia Type III

Congenital dyserythropoietic anemia type III is an extremely rare condition and has been observed in both familial and sporadic forms (Table 23.3) [55]. The familial form is autosomal dominant. CDA type III is characterized by a mild to moderate macrocytic anemia, bone marrow erythroid hyperplasia, and the presence of dysplastic, giant erythroid precursor cells with one or multiple nuclei [56, 57]. The erythrocytes may react with anti-I and/or anti-i sera, but serum acid test is negative. Linkage analysis and recombination studies in a Swedish family have suggested a gene (*CDAN3*) located in the region of chromosome 15q22 [55].

Other Types of Congenital Dyserythropoietic Anemia

Several forms of CDA other than types I, II, and III have been reported. These briefly include the following.

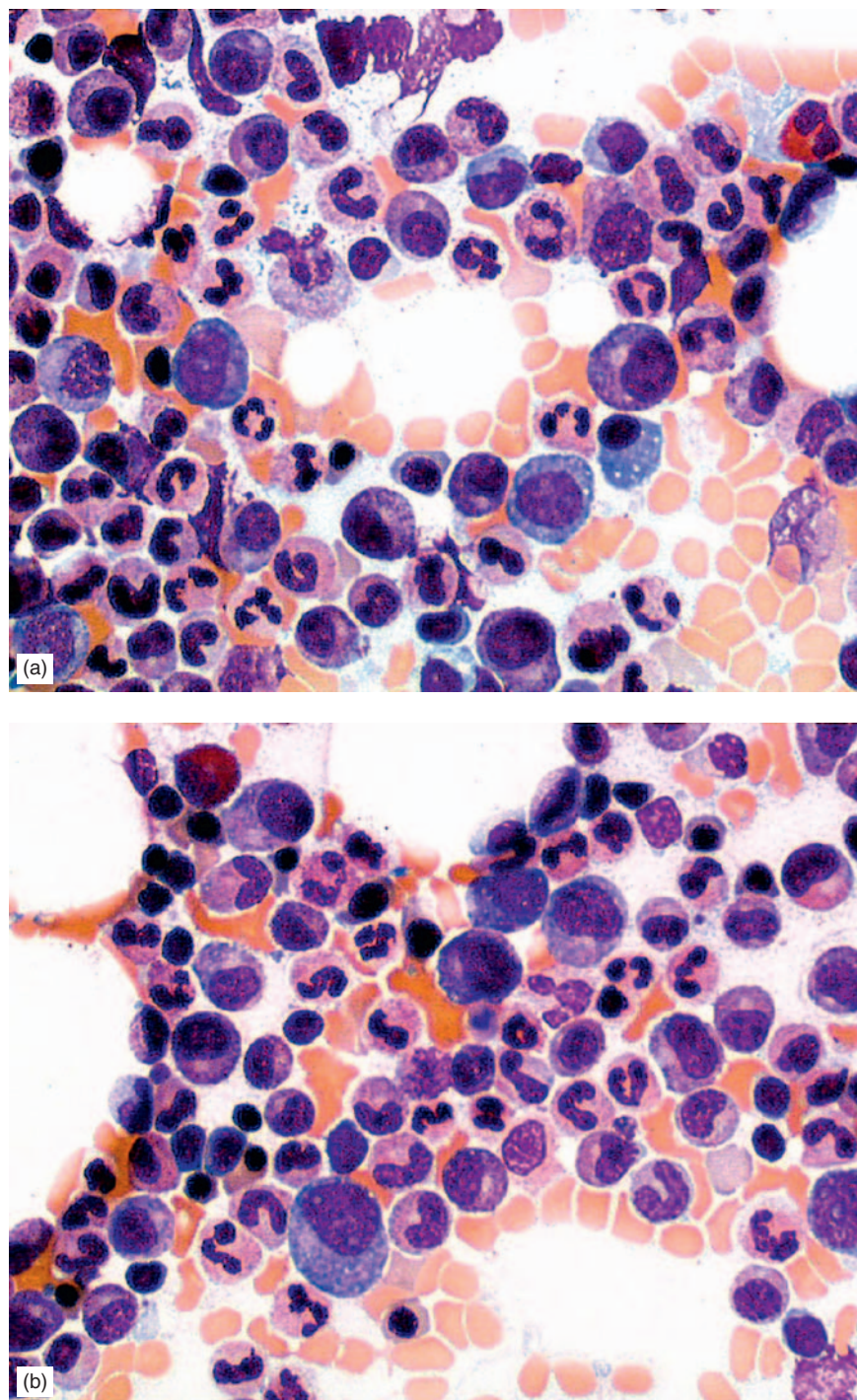


FIGURE 23.10 Bone marrow smears of a patient with pure red cell aplasia (a and b) showing marked myeloid preponderance and occasional erythroid precursors.

CDA group IV is characterized by severe transfusion-dependent anemia since birth with absence of precipitated protein in erythroblasts [37].

CDA group V represents patients with congenital ineffective erythropoiesis with insignificant dysplasia, mild anemia, and elevated unconjugated bilirubin [58].

CDA group VI is characterized by marked congenital macrocytosis and folate- and vitamin B₁₂-independent megaloblastic erythropoiesis with mild anemia [37].

Differential Diagnosis

The differential diagnosis includes all congenital and acquired anemias known to be associated with dyserythropoiesis, such as β -thalassemia, hereditary sideroblastic anemias, PK deficiency anemia, myelodysplastic syndromes, megaloblastic anemia, parvovirus B19 infection, arsenic poisoning, and severe IDA [37, 59]. Diagnosis of CDA should

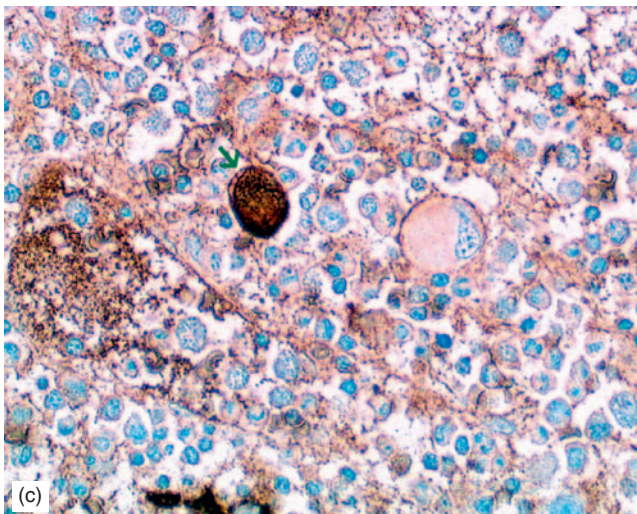
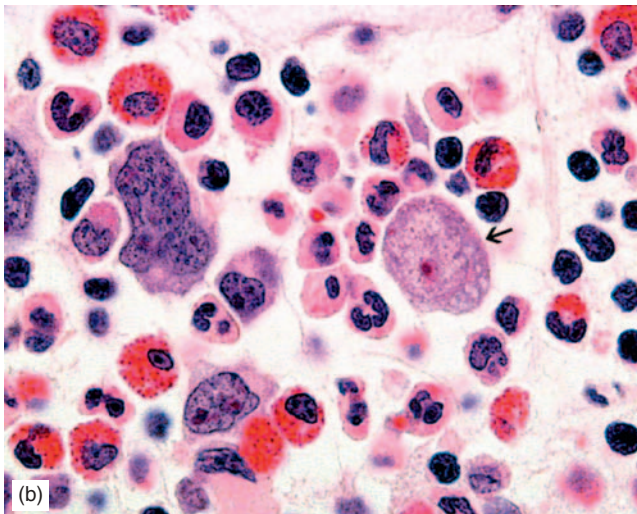
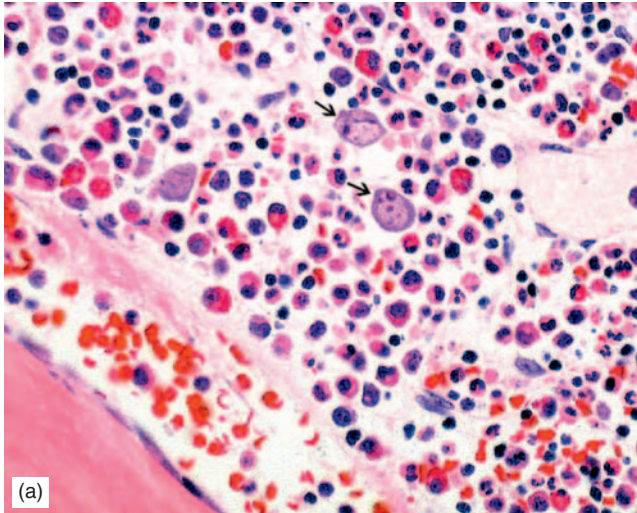


FIGURE 23.11 Pure red cell aplasia caused by parvovirus B19 infection. Bone marrow biopsy section demonstrates myeloid preponderance, eosinophilia, and scattered giant early erythroid precursors: (a) low power and (b) high power (arrows). A parvovirus-positive cell is shown by immunohistochemical stain (c).

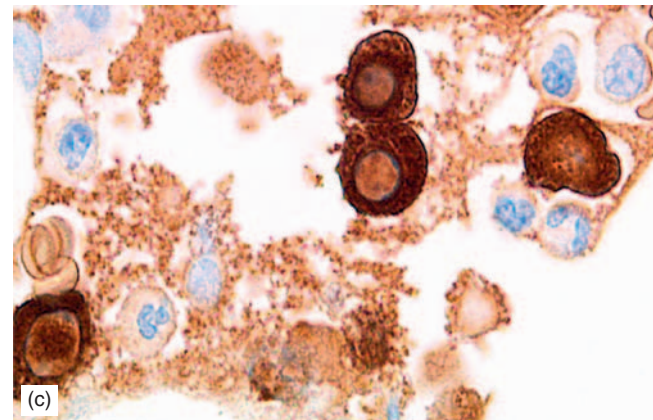
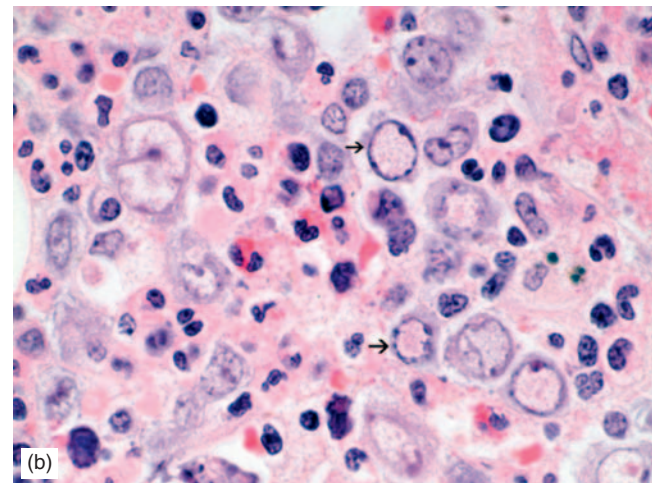
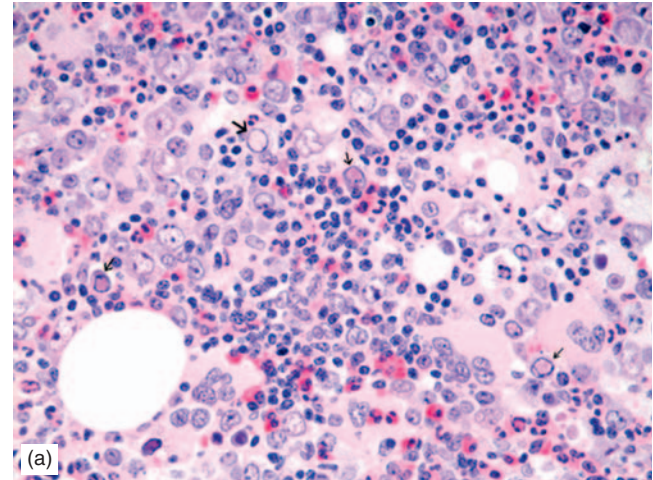


FIGURE 23.12 Parvovirus B19 infection. Bone marrow biopsy section demonstrates several early erythoid cells with nuclear inclusions: (a) low power and (b) high power (arrows). Several parvovirus-positive cells are shown by immunohistochemical stain (c).

be considered when the reticulocyte count does not correlate with the degree of anemia in a patient with erythroid hyperplasia or when there is unexplained hyperbilirubinemia or iron overload [37].

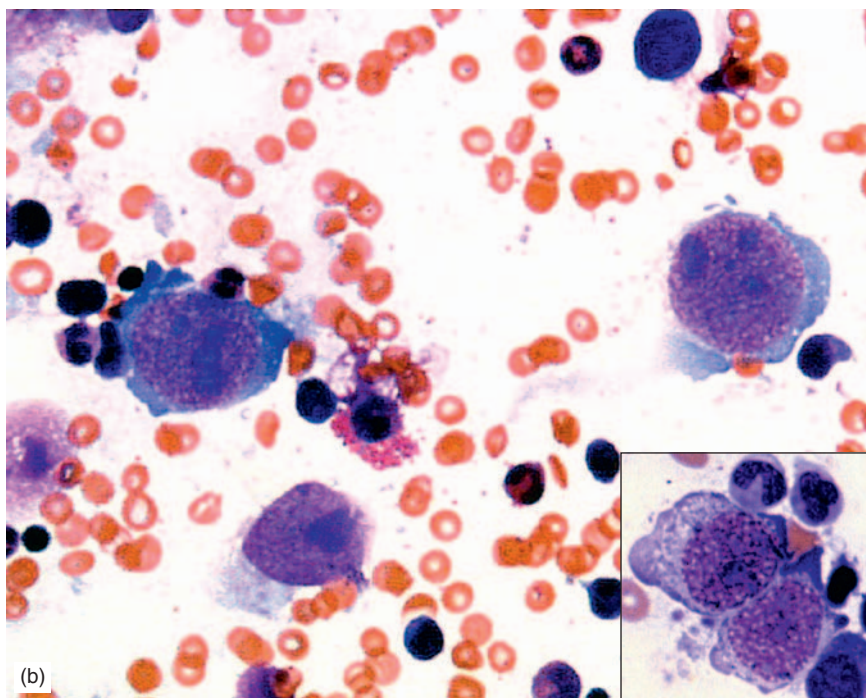
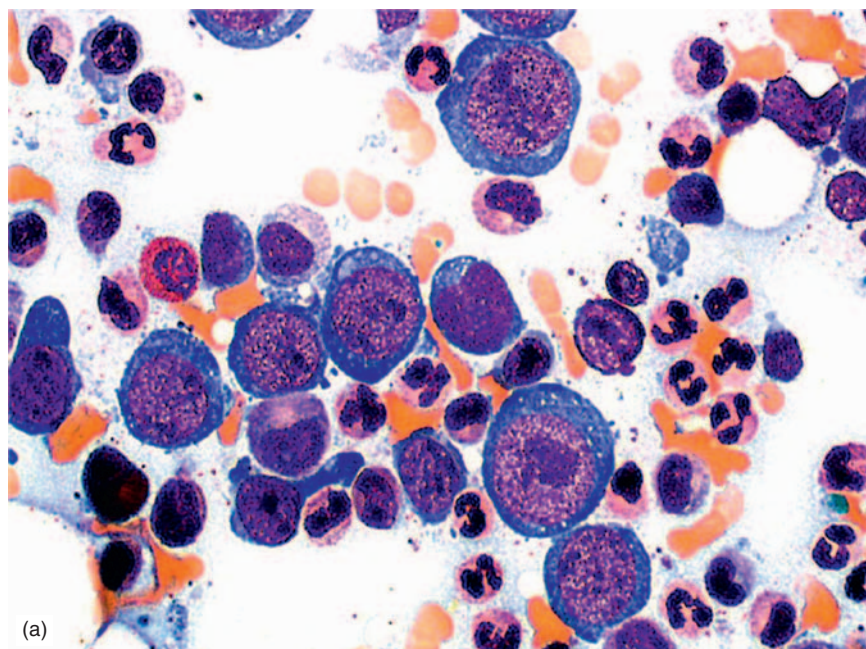


FIGURE 23.13 Bone marrow smear (a, b, and inset) demonstrates several large erythroblasts in a patient infected with parvovirus B19.

MACROCYTIC ANEMIAS

Macrocytic anemias are a group of anemias in which the RBCs are larger than normal (MCV >100 fl). Macrocytic anemia is caused by various disorders such as folate and vitamin B₁₂ deficiencies, alcoholism, liver disease, hypothyroidism, and myelodysplastic syndromes (Table 23.4). In general, macrocytic anemia can be divided into two major categories: (1) megaloblastic anemias and (2) non-megaloblastic anemias. In this chapter, megaloblastic anemias are discussed.

Megaloblastic Anemias

Megaloblastic anemias are a group of anemias characterized by megaloblastic erythropoiesis and macrocytosis.

Etiology and Pathogenesis

The underlying defect in megaloblastic anemia is the decline in the rate of DNA synthesis leading to a delay in cell division in all proliferating cells. This defect is due to abnormal purine or pyrimidine metabolism, or inhibition of DNA

TABLE 23.3 Features of the major types of CDA.*

Features	Type I	Type II	Type III
Inheritance	Autosomal recessive	Autosomal recessive	Autosomal dominant or recessive
Gene; chromosome	<i>CDAN1</i> ; 15q15.1-15.3	<i>CDAN2</i> ; 20q11.2	<i>CDAN3</i> ; 15q22
Red cells	Macrocytic	Normocytic	Large macrocytic
Erythroblasts	Megaloblastic; nuclear chromatin bridges	Normoblastic; binucleated cells	Megaloblastic; giant mono- or multinucleated cells
Ham test	Negative	Usually positive	Negative
Glycosylation	Some abnormality	Markedly abnormal	Some abnormality

*Adapted from Ref. [37].

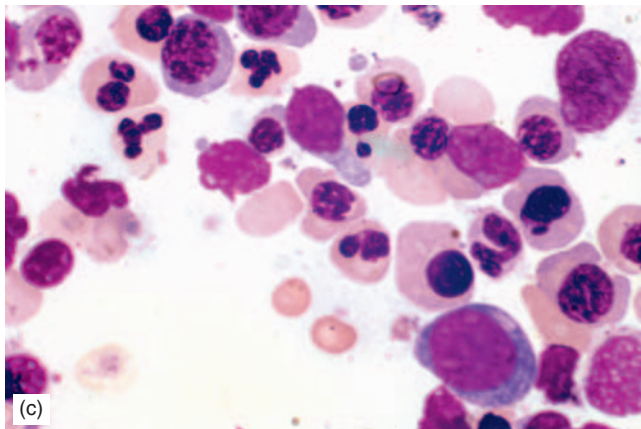
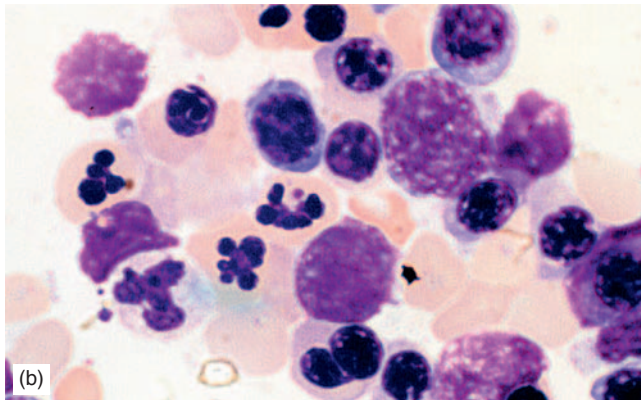
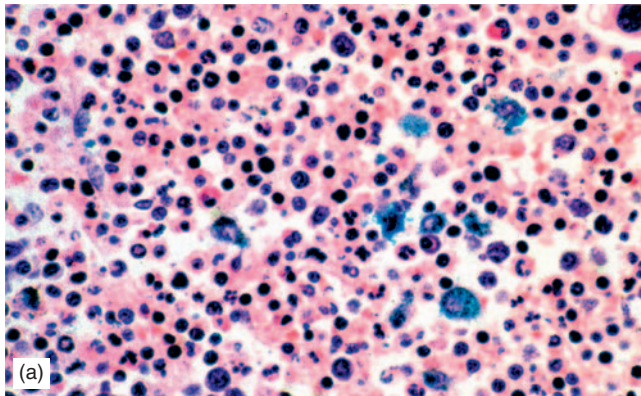
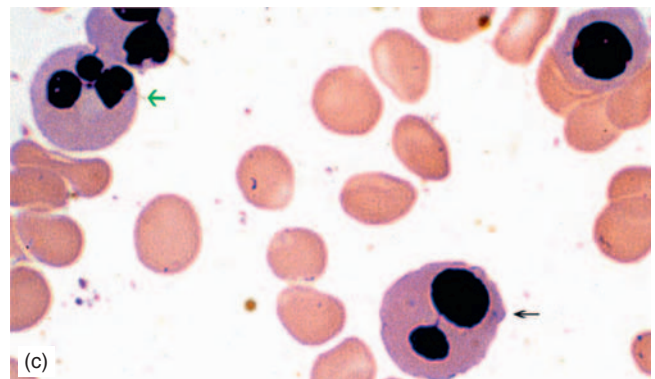
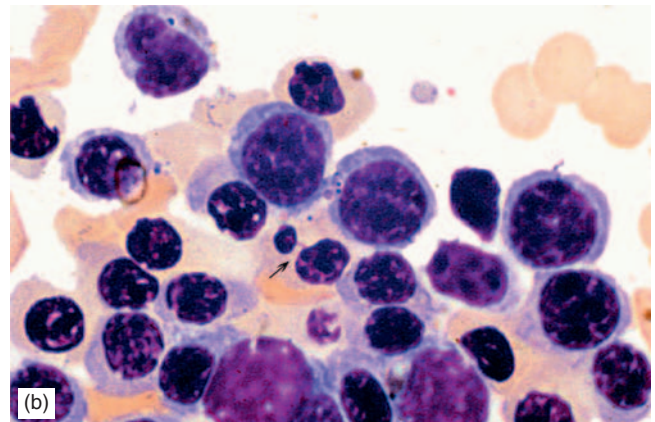
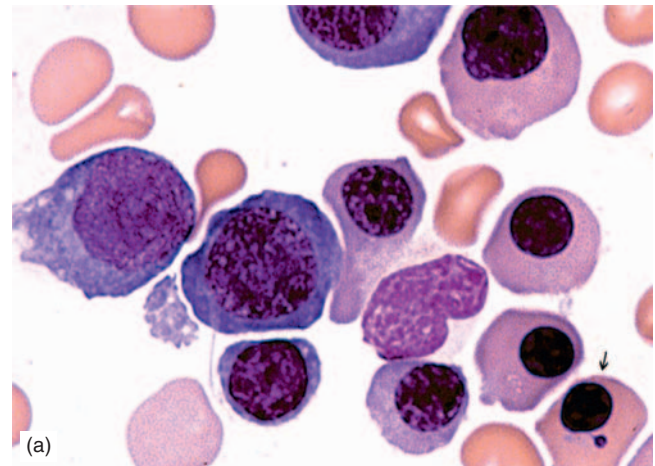
**FIGURE 23.14** Congenital dyserythropoietic anemia. Bone marrow biopsy (a) and smear (b and c, arrows) demonstrate numerous intermediate and late-stage erythroid precursors with lobulated nuclei.**FIGURE 23.15** Congenital dyserythropoietic anemia. Bone marrow smear (a, b, and c) shows double segmented nuclei, each segment in different size (black arrows). A dysplastic, multilobated erythroid precursor is shown (c, green arrow).

TABLE 23.4 Common causes of macrocytosis.*

Newborn
Alcoholism
Reticulocytosis
Folate deficiency
Cobalamin (vitamin B ₁₂) deficiency
Liver disease
Hypothyroidism
Myelodysplastic syndromes
Aplastic anemia
Hairy cell leukemia
Acute leukemia
Drugs
Chemotherapeutic (e.g. cyclophosphamide, methotrexate)
Antiviral (e.g. zidovudine, stavudine)
Hypoglycemic (metformin)
Antimicrobial (e.g. sulfamethoxazole, valacyclovir)
Diuretics (furosemide)
Anticonvulsant (e.g. phenytoin, primidone)
Anti-inflammatory (sulfasalazine)

*Adapted from Ref. [62].

polymerization [60–64]. The major etiologic factors in megaloblastic anemia are (1) dietary insufficiency and acquired or congenital conditions which lead to folate (folic acid) or cobalamin (vitamin B₁₂) deficiencies and (2) congenital or acquired defects of purine or pyrimidine metabolism.

Folate Deficiency

Folate is primarily absorbed through the upper third of the small intestine and is transported to the cells mainly as 5-methyl tetrahydrofolate (5M-THF). THF is required in the methylation of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) in the presence of thymidylate synthetase [61, 65, 66]. Folate deficiency leads to decreased synthesis of dTMP and increased levels of dUMP, and this imbalance apparently slows down DNA synthesis and delays cell proliferation [67, 68]. The end result of this retardation in DNA synthesis is inappropriate enlargement of the cells (megaloblasts), which contain more than the normal amount of DNA but not enough for cell division [60].

Folate deficiency is caused by decreased intake, malabsorption, excess loss, increased requirements, drug effects, and metabolic defects (Table 23.5). The major cause of folate deficiency is dietary. Folate is heat labile and is present in various green vegetables, yeast, mushrooms, kidney, and liver.

Vitamin B₁₂ Deficiency

Vitamin B₁₂ is involved in the conversion of 5M-THF to THF, which is required for the methylation of dUMP to dTMP. Vitamin B₁₂ deficiency, similar to folate deficiency, leads to disturbance of DNA synthesis, and consequently,

TABLE 23.5 Major causes of folate deficiency.*

1. Decreased intake
a. Malnutrition (e.g. poverty, old age, alcoholism)
b. Special diet (e.g. goat's milk)
c. Hyperalimentation
2. Malabsorption
a. Gluten-induced
b. Tropical sprue
c. Other diseases of small intestine
3. Excess loss of folate
a. Dialysis
b. Congestive heart failure
4. Increased requirement
a. Pregnancy
b. Premature infants
c. Excessive marrow turnover (e.g. hemolytic anemia)
d. Inflammatory diseases (e.g. rheumatoid arthritis, exfoliative dermatitis)
e. Cancers
5. Drugs
a. Folate antagonists (e.g. methotrexate)
b. Anticonvulsants
c. Barbiturates
6. Congenital defects
a. Congenital folate malabsorption
b. Dihydrofolate reductase deficiency
c. Homocysteine methyltransferase deficiency

*Adapted from Naeim F. (1998). *Pathology of Bone Marrow*, 2nd ed. Williams & Wilkins, Baltimore.

megaloblastic anemia [60, 69–71]. Vitamin B₁₂ is heat resistant and is abundant in animal proteins. The most common cause of vitamin B₁₂ deficiency is impaired intestinal absorption. Other less frequent causes include inadequate intake and metabolic defects (Table 23.6).

In the digestive system, vitamin B₁₂ attaches to salivary and gastric vitamin B₁₂ binders (R binders; haptocorrins). The R-vitamin B₁₂ complexes are broken when exposed to pancreatic enzymes and the R binders are digested. The released vitamin B₁₂ binds to the intrinsic factor (IF), a glycoprotein secreted by the gastric parietal cells [72]. The IF–vitamin B₁₂ complex is then carried to the ileum, where it binds to specific receptors. The IF is then separated from vitamin B₁₂, and the free vitamin B₁₂ is absorbed. The absorbed vitamin B₁₂ enters the portal circulation and binds to transcobalamins, mainly transcobalamin II. IF deficiency leads to megaloblastic anemia. Megaloblastic anemia secondary to IF deficiency is known as *pernicious anemia* [73].

Pernicious anemia is either congenital or acquired. The congenital form is a rare disorder characterized by mutations in the *IF* gene localized on chromosome 11q13 and lack of IF production. In congenital IF deficiency, gastric acid secretion and mucosal cytology are normal [74–76]. The acquired pernicious anemia, in most cases, is an autoimmune disorder characterized by chronic atrophic gastritis and reduced IF production due to the presence of autoantibodies against gastric parietal cells [73, 77].

TABLE 23.6 Major causes of vitamin B₁₂ deficiency.*

1. Impaired absorption
a. Gastric origin
i. Pernicious anemia
ii. Zollinger–Ellison syndrome
iii. Gastrectomy, partial or total
b. Intestinal origin
i. Iliac disease or resection
ii. Blind loop syndrome
iii. Chronic tropical sprue
iv. Fish tapeworm
v. Drugs (e.g. metformin)
2. Inadequate diet
3. Metabolic defects
a. Congenital
i. Transcobalamin II deficiency
ii. Homocystinuria and methylmalonic aciduria
iii. Hereditary orotic aciduria
iv. Lesch–Nyhan syndrome
b. Acquired
i. Anesthesia with nitrous oxide
ii. Drug-induced
4. Others (e.g. congenital R-binding deficiency)

*Adapted from Naeim F. (1998). *Pathology of Bone Marrow*, 2nd ed. Williams & Wilkins, Baltimore.

Disturbance of Purine and Pyrimidine Metabolism

The defects of purine and pyrimidine metabolism are either acquired or congenital. The major causes of acquired defects are antimetabolite drugs (purine and pyrimidine analogs). *Hereditary orotic aciduria* and *Lesch–Nyhan syndrome* represent the congenital deficiencies. Hereditary orotic aciduria is an autosomal recessive disorder of pyrimidine metabolism characterized by orotidyl decarboxylase deficiency [63, 78, 79]. Lesch–Nyhan syndrome is an X-linked disorder caused by a deficiency of hypoxanthine-guanine phosphoribosyl-transferase [80].

Pathology and Laboratory Studies

The impaired DNA synthesis in megaloblastic anemia slows nuclear replication and cell division and leads to ineffective erythropoiesis and premature destruction of the RBCs. Bone marrow sections and smears are hypercellular and reveal erythroid hyperplasia. There is often a shift to the left with numerous megaloblasts. Megaloblastic erythroid precursors are larger than their normal counterparts, show asynchronous nuclear and cytoplasmic maturation, display coarser nuclear chromatin, and have more cytoplasm relative to the size of the nucleus (Figures 23.16–23.18). These megaloblastic features are more striking in the intermediate and late stages of erythroid maturation, demonstrated as unevenly speckled nuclear chromatin and abundant Hb-loaded cytoplasm [81]. Final condensation of chromatin (pyknosis), which is seen in late orthochromatic normoblasts, is either delayed or fails to occur. Dysplastic changes such as nuclear fragmentation and nuclear irregularity or lobulation are common. The megaloblastic changes and the

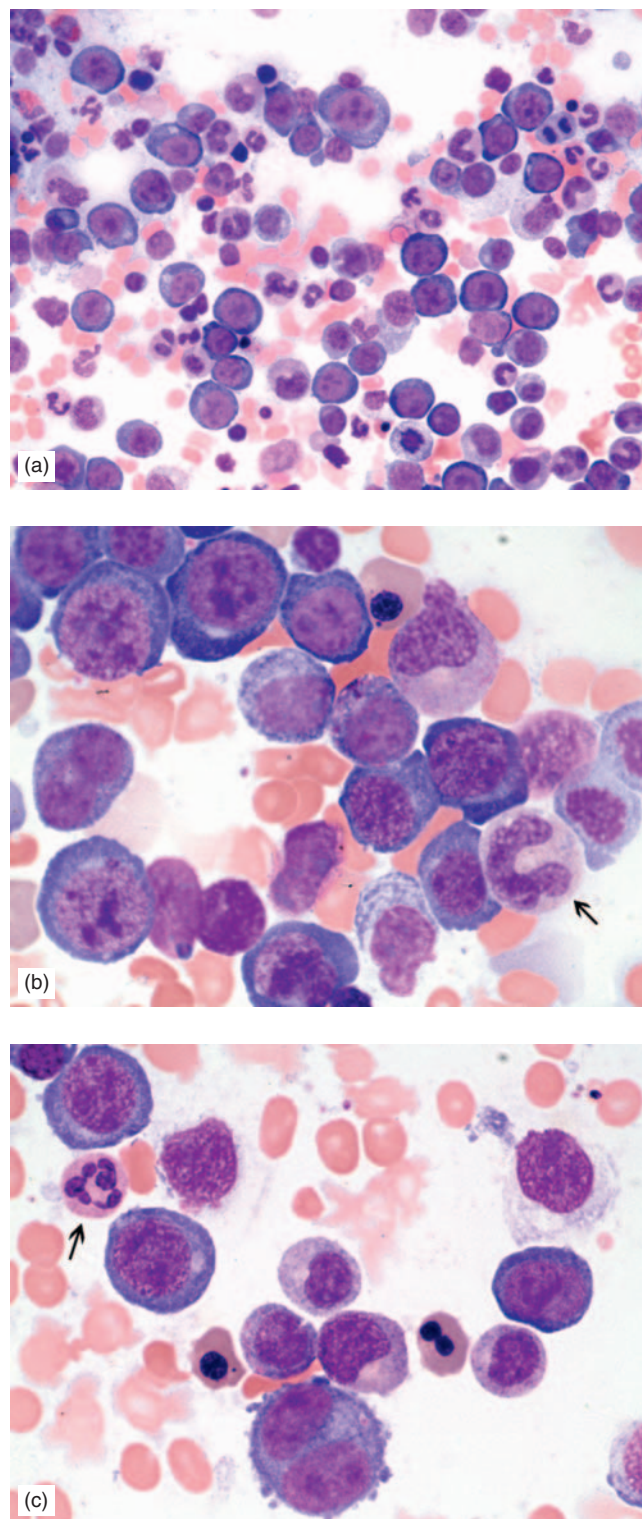


FIGURE 23.16 Megaloblastic anemia. Bone marrow smear demonstrating megaloblastic erythroid precursors (a and b) with a giant band (b, arrow); (c) shows a hypersegmented neutrophil (arrow), two binucleated erythroid precursors, and one with a small nuclear fragment.

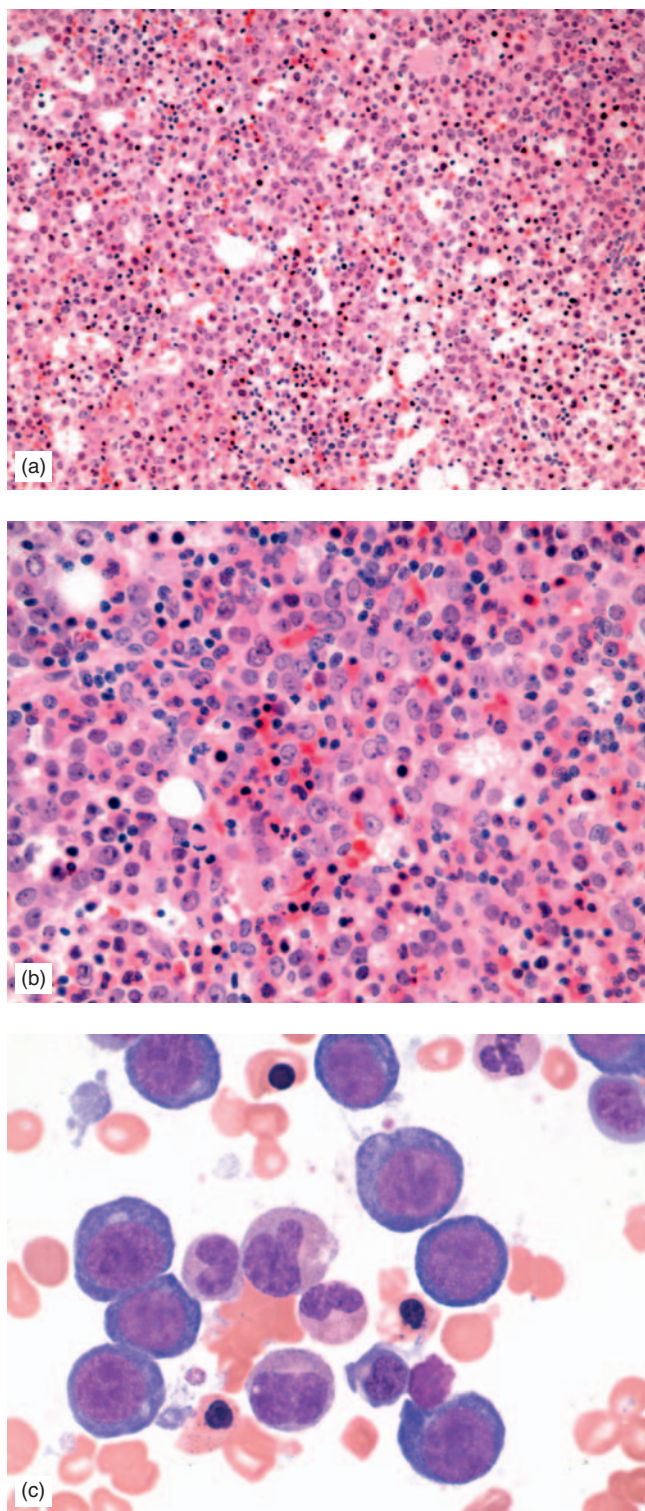


FIGURE 23.17 Megaloblastic anemia. Biopsy section demonstrates a hypercellular marrow with a high proportion of large early erythroid precursors: (a) low power and (b) high power views. Bone marrow smear shows numerous megablasts and scattered late erythroid precursors with irregular nuclei (c).

erythroid left shift may simulate an acute leukemic process, particularly in H&E sections, where clusters of immature erythroid cells with open nuclear chromatin and prominent nucleoli are found.

The granulocytic series are also affected and show nuclear-cytoplasmic asynchrony with the presence of giant metamyelocytes and bands, and hypersegmented neutrophils. Mild to moderate myeloid left shift is a common feature. Megakaryocytic lineage may display nuclear hypersegmentation.

Peripheral blood examination reveals pancytopenia with increased MCV of usually >115 fl. Smears show anisopoikilocytosis, macro-ovalocytes, and hypersegmented neutrophils (five nuclear segments in $>5\%$ of neutrophils or more than six nuclear segments in $>1\%$ of neutrophils) [82]. Other features include basophilic stippling, Howell-Jolly bodies, and occasionally, Cabot rings. In severe cases, numerous nucleated RBCs are present. Coincident iron deficiency or thalassemia can mask macrocytosis [83].

Laboratory studies reveal reduced concentrations of serum and RBC folate in patients with megaloblastic anemia due to folate deficiency. Pregnancy, alcohol intake, and certain anticonvulsant drugs may cause a decrease in serum levels despite adequate tissue stores [62, 84]. Hemolysis may falsely elevate serum folate levels due to high concentration of folate in RBCs [62, 85].

Different laboratory methods are used for the measurement of serum vitamin B₁₂ levels. Serum vitamin B₁₂ levels may be falsely low in pregnancy, use of oral contraceptives, congenital deficiency of serum haptocorrins (R binders), and plasma cell myeloma [62, 85]. The Schilling test is performed to evaluate vitamin B₁₂ absorption and to distinguish various causes of its reduced absorption, such as pernicious anemia/gastrectomy, iliac disease, or intestinal bacterial over growth [86].

Folate and vitamin B₁₂ are both required for the conversion of homocysteine to methionine. Therefore, their deficiencies may lead to the elevation of serum homocysteine concentration and increased risk of atherosclerosis and venous thromboembolism [83, 87]. Serum methylmalonic acid levels are elevated only in patients with vitamin B₁₂ deficiency [62, 88, 89].

Clinical Aspects

Folate and vitamin B₁₂ deficiencies are the second and third most common causes of nutritional anemia in the world, respectively (iron deficiency is the first) [83, 90]. The major difference in the clinical manifestation between these two deficiencies is that only B₁₂ deficiency manifests neurologic symptoms. The vitamin B₁₂ stores in body are so large relative to the daily requirements that it takes years of inadequate supply before clinical symptoms develop. On the contrary, symptoms of megaloblastic anemia due to folate deficiency can occur within a few months after its supply is diminished [83].

The most common cause of folate deficiency is nutritional due to poor diet or alcoholism. The normal daily requirement of folate is about 200–400 $\mu\text{g}/\text{day}$, but it increases to 500–800 $\mu\text{g}/\text{day}$ during pregnancy and lactation. The most frequent cause of vitamin B₁₂ deficiency is

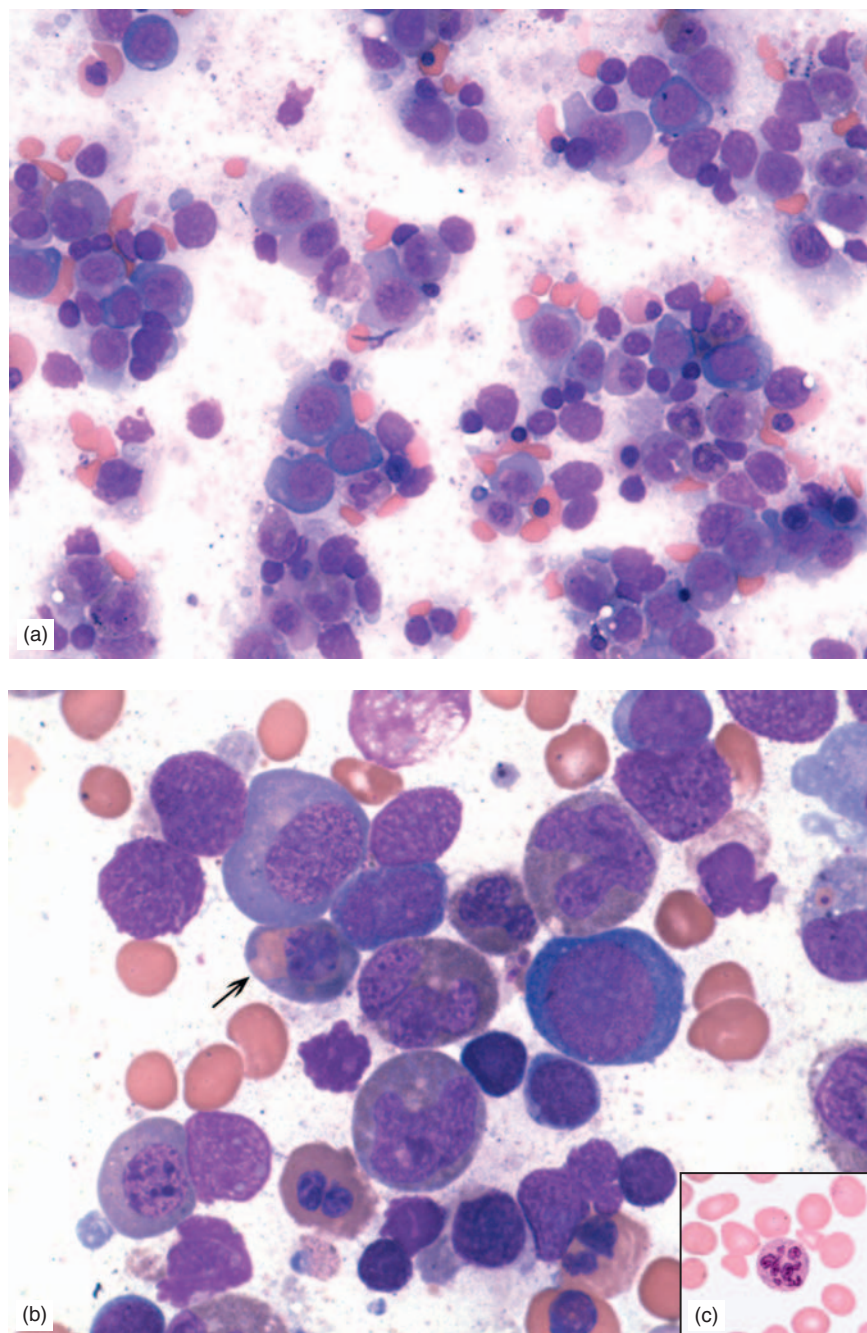


FIGURE 23.18 Megaloblastic anemia. Bone marrow smear demonstrates erythroid preponderance with a high proportion of megaloblasts: (a) low power and (b) high power views. An unevenly hemoglobinized erythroid cell is shown by an arrow (b). Blood smear shows macro-ovalocytes and a hypersegmented neutrophil (c).

inadequate absorption due to IF deficiency (pernicious anemia), gastric disease, or gastrectomy. The minimum dietary requirement of vitamin B₁₂ is 6–9 µg/day. Unlike folate deficiency, vitamin B₁₂ deficiency may be associated with neurologic symptoms. These symptoms result from degenerative changes of the dorsal and lateral columns of the spinal cord (subacute combined system disease) and have been attributed to the impairment of myelin synthesis due to the accumulation of methylmalonyl CoA or impairment of methyl group metabolism [91, 92].

A potentially fatal megaloblastic anemia due to a rapid tissue folate depletion has been observed in association with nitrous oxide (N₂O) anesthesia [93].

With the administration of folate or vitamin B₁₂, reticulocyte count will be increased in 3–4 days, followed by a fall in MCV and a rise in Hb levels within 10 days.

Hypersegmented neutrophils disappear in 10–14 days and Hb levels return to normal within 8 weeks [81].

Differential Diagnosis

Megaloblastic changes are observed in various conditions such as myelodysplastic syndromes, erythroleukemia, CDA, parvovirus infection, and following chemotherapy. In these conditions, serum folate and vitamin B₁₂ levels are elevated or within normal limits. Increased number of early erythroid

precursors and the presence of myeloid left shift in bone marrow biopsy sections may sometimes resemble acute leukemia. Immunophenotypic studies and review of the bone marrow smears will help to identify the erythroid nature of the immature cells. Coexistence of megaloblastic anemia with microcytic anemias, such as IDA or thalassemia, may mask macrocytosis, but hypersegmented neutrophils and giant bands are still present.

MICROCYTIC ANEMIAS

Microcytic anemias are anemias with red cells smaller than normal ($MCV < 80$ fl). The most common cause of microcytic anemia is iron deficiency followed by thalassemias. Anemia of chronic disease, sideroblastic anemia, copper deficiency, and zinc intoxication may also be associated with microcytic anemia.

Iron Deficiency Anemia

Iron deficiency anemia (IDA) is the most common anemia worldwide [94]. The highest frequency of IDA is seen in women of reproductive age, pregnant women, and premature infants.

Etiology and Pathogenesis

Iron is usually lost through blood loss or loss of cells as they slough [94]. Major causes of IDA are inadequate dietary intake, blood loss, hemoglobinuria, iron malabsorption, renal dialysis, or inability of erythroid precursors to utilize iron [94–98].

Inadequate dietary intake is seen in infants fed milk without supplementary iron [99, 100]. Iron is low in milk and milk products, and prolonged (>6 months) breast or bottle feeding without iron supplementation may lead to IDA. Premature infants may become iron deficient as early as 10–12 weeks after birth, if their diet is not supplemented with iron. Iron dietary requirements increase during pregnancy and lactation.

Blood loss, particularly chronic bleeding, is one of the major causes of iron deficiency in adults [101, 102]. Chronic gastrointestinal bleeding due to peptic ulcer, gastritis, ulcerative colitis, amebiasis, hiatus hernia, esophageal and gastric varices, and cancers are the most common causes of IDA in men and post-menstrual women [94, 101, 103]. Bleeding of genitourinary tract, such as hematuria, hemoglobinuria (e.g. paroxysmal nocturnal hemoglobinuria, PNH), menstruation, and menorrhage often leads to the depletion of iron store. Similarly, IDA is observed in respiratory tract blood loss secondary to infection, epistaxis, idiopathic pulmonary hemosiderosis, and Goodpasture syndrome [103–105].

Iron deficiency is associated with the depletion and disappearance of hemosiderin and ferritin from bone marrow and other storage sites, and decreased concentration of several iron-containing proteins such as Hb, myoglobin, cytochrome c, cytochrome oxidase, and xanthine oxidase [106]. Approximately 1–2 mg/day iron is absorbed through

the digestive system. Iron absorption is retarded by phosphate and phytates from cereals and is enhanced by vitamin C [107]. Meat, eggs, and liver are rich in iron. All vegetables except legumes and all fruits are either poor in iron or contain unabsorbable iron chelates.

Pathology and Laboratory Studies

Bone marrow examination often reveals mild to moderate hypercellularity with erythroid preponderance. Erythropoid precursors, particularly intermediate and late normoblasts, are small and show scanty, ragged rims of poorly hemoglobinized cytoplasm (Figure 23.19). These morphologic findings are not consistent and do not correlate with the severity of anemia. Bone marrow hemosiderin (demonstrated by Prussian blue stain) is reduced or absent. However, lack of stainable iron has been noted in patients with chronic myelogenous leukemia and myelofibrosis. These patients usually lack other evidences of iron deficiency.

Peripheral blood examination in the early stages of IDA reveals mild anisocytosis with slightly elevated red cell distribution width (RDW) and decreased MCV (<80 fl) (Figure 23.19). In more severe forms of IDA, erythrocytes are clearly microcytic and hypochromic and show pronounced anisocytosis with the presence of target cells.

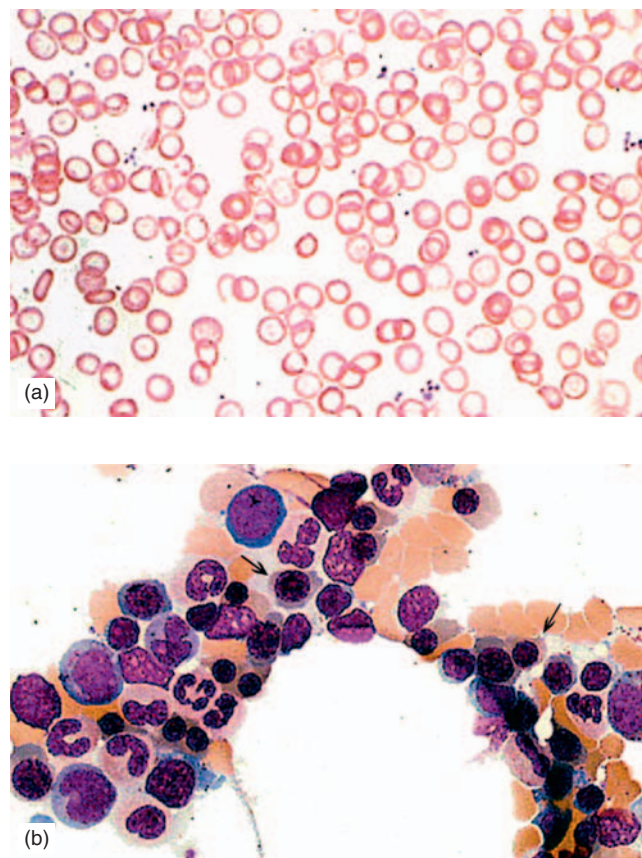


FIGURE 23.19 Iron deficiency anemia. Blood smear demonstrates hypochromic microcytic red cells (a) and bone marrow smear shows erythroid precursors with ragged rims (b, arrows).

The RDW is markedly elevated, and the MCV, MCH, and Hb levels are significantly lower than the normal range. Serum iron and ferritin levels are low and serum transferrin or total iron-binding capacity (TIBC) is elevated, as is the concentration of erythrocyte protoporphyrin (Table 23.7).

Serum ferritin is invariably low in IDA, but it is elevated in anemia of chronic disease, sideroblastic anemia, and thalassemia (Table 23.8) [108–110].

Clinical Aspects

Symptoms of anemia such as fatigue, headache, paresthesia, and burning sensation of the oropharyngeal mucosa are often preceded by the depletion of iron stores [94]. There is a poor correlation between the severity of the symptoms and the blood Hb level, suggesting that some of the symptoms are caused by a deficiency of iron-containing enzymes or proteins rather than by a low concentration of Hb. *Pica* (appetite for substances not considered as food, such as clay or dirt) and *pagophagia* (pica for ice) may be early clinical symptoms [111, 112].

Impaired learning ability and growth in children, defects in cell-mediated immunity and bactericidal function of leukocytes, increased frequency of premature contractions during pregnancy, and possibly an increased rate of premature births have been reported in patients with iron deficiency [113–115]. Most of the symptoms subside a few days after initiation of iron therapy. The reticulocyte count reaches its peak at about 1–2 weeks and then gradually levels off. The Hb level begins to improve after 2 weeks and usually comes back to normal levels after 2 months of adequate iron therapy.

TABLE 23.7 Laboratory findings in iron deficiency anemia.

Parameter	Results
Hemoglobin	<13 g/dL in men <12 g/dL in women
MCV	<80 fL
Serum iron	<75 µg/dL in men <65 µg/dL in women
Transferrin (TIBC)	<450 µg/dL
Transferrin saturation	<16%
Serum ferritin	<10 µg/L
Erythrocyte protoporphyrin	>7 µg/dL RBC
Bone marrow iron stain	Markedly reduced or negative

Thalassemia Syndromes

Thalassemia syndromes are a group of disorders caused by inherited defects in the synthesis of one or more of the globin chains and are characterized by a microcytic anemia [116–118]. The defect in globin chain production leads to a change in the proportion of the Hb classes, which consists of Hb A ($\alpha_2\beta_2$), Hb A2 ($\alpha_2\delta_2$), and Hb F ($\alpha_2\gamma_2$), constituting >95%, >3%, and <2% of the Hb molecules in normal adults, respectively.

The main β - and α -globin gene clusters are located on chromosomes 11 and 16, respectively (Figure 23.20), and encode globin subunits specific for the embryonic, fetal, and adult developmental stages [119–122]. In normal conditions, a balance is maintained between α - and β -cluster gene expression so that functional Hb tetramers are assembled. In thalassemia this balance is lost [120, 121]. The reduction in the synthesis of certain globin chain(s) leads to relative excess of the non-affected globin chain(s), causing Hb instability and hemolysis. Thalassemia syndromes are divided into β , δ , $\beta\delta$, and α . δ -Thalassemia has no clinical significance.

β -Thalassemia

β -Thalassemia is the result of impaired production of β -chains of Hb A, leading to excess α -globin chains [117, 123–126]. Excess α -globin chains are unstable and precipitate in the erythrocytes (Heinz bodies) leading to the disruption of cell membrane and hemolysis. The excess α -chain deposition also accelerates apoptosis, leading to shortened red cell survival and ineffective erythropoiesis [127]. The exact mechanism of apoptosis is not clear, but a death-receptor-mediated pathway with Fas–Fas interactions has been suggested [128]. The abnormal β -globin gene expression is primarily the result of point mutations, but it may also be due to deletion of long stretches of nucleotides, or substitution of a small number of nucleotides within or close to the β -globin gene [119]. More than 200 point mutations have been reported [123], but ethnic-specific mutations allow for practical testing using more limited panels. The principal techniques used are DNA sequencing and allele-specific probe hybridization. Other genetic changes are less frequent. β -Thalassemia has two major genotypic types: homozygous with both β -genes affected (β_0) and heterozygous with one β -gene involved (β_+). There is a considerable phenotypic variation, depending on the nature of the mutations and the extent of the affected genes.

TABLE 23.8 Clinicopathologic features of common β -thalassemia syndromes.*

Features	Thalassemia trait	Thalassemia intermedia	Thalassemia major
Genetic	Heterozygous with one mutated β -globin gene	Heterozygous with more than one mutation in β -globin genes, one of which being partially defective	Homozygous, two β -globin genes affected
Clinical	Mild microcytic anemia; no other clinical symptoms	Moderate anemia, splenomegaly, and iron overload	Severe transfusion-dependent anemia, splenomegaly, iron overload, bone deformities

*Adapted from Ref. [121].

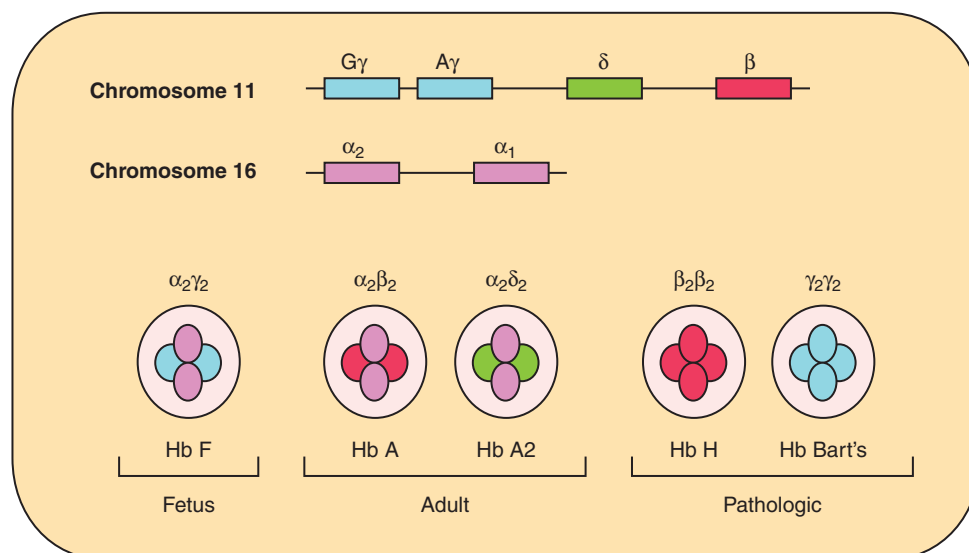


FIGURE 23.20 Hb genes are located on chromosomes 11 and 16. Normal and abnormal Hb structures are demonstrated.

Pathology and Laboratory Studies

Pathologic findings correlate with the severity of the clinical manifestations. In general, symptomatic patients show a hypercellular bone marrow with marked erythroid hyperplasia and left shift with some megaloblastic features (Figure 23.21). The iron stores are increased with abundant iron-laden macrophages. Scattered macrophages may contain phagocytosed normoblasts. The polychromatophilic normoblasts are poorly hemoglobinized, show ragged cytoplasm, and contain precipitated α -globin chains. The severely affected patients may show extramedullary erythropoiesis with masses of erythroid colonies in the thoracic or pelvic bone marrow spaces, causing spinal cord compression or other symptoms [126, 129].

The peripheral blood shows marked microcytic hypochromic anemia with low MCV and MCH, and elevated RDW and reticulocyte count (Figure 23.21). Anisocytosis is prominent, and there is a variable degree of poikilocytosis, target cell formation, and basophilic stippling. Heinz bodies may be detected in the affected RBCs using supravital stains such as methyl violet. Nucleated RBCs are often present. The WBC count is usually elevated but the platelet count is within normal limits. The serum iron and ferritin levels are elevated. The transferrin saturation rate (ratio of serum iron to transferrin) is also high. Sera of the affected patients usually demonstrate increased concentration of indirect (unconjugated) bilirubin, elevated levels of lactate dehydrogenase (LDH), and reduced haptoglobin concentration, all indicative of hemolysis.

One of the characteristic features of thalassemia major (β_0 -thalassemia) is elevated levels of fetal Hb. The proportion of Hb A2 to Hb A is also elevated. Hb electrophoresis (alkaline cellulose acetate and acidic citrate agar) is the most widely used method for diagnosis of hemoglobinopathies. However, newer techniques such as high-performance liquid chromatography (HPLC) provide higher sensitivity and specificity (Figure 23.22) [130, 131]. Isoelectric focusing provides excellent resolution but lacks quantitative accuracy and is labor intensive [130].

Clinical Features

β -Thalassemia syndromes are widespread in the Mediterranean basin, Middle East, and Southeast Asia with a spectrum of clinical manifestations ranging from a very mild asymptomatic anemia to severe transfusion-dependent anemia [121, 129, 132]. These syndromes are divided into three main categories: (1) β -thalassemia minor, (2) β -thalassemia major, and (3) β -thalassemia intermedia (Table 23.8).

β -Thalassemia minor, also known as β -thalassemia trait, is referred to heterozygous status when only one β -globin gene is affected. Patients with β -thalassemia trait are asymptomatic and may show mild anemia, usually detected as an incidental finding by a routine blood examination. Anemia may get worse during stressful conditions such as severe infection or pregnancy [133, 134]. The Hb levels are about 9–11 g/dL, but the MCV and MCH are markedly reduced.

β -Thalassemia major is referred to the patients who are homozygote and have no ability for effective production of β -globin. The disease, also known as Cooley's anemia, usually starts during the first year of life with profound transfusion-dependent anemia (Hb as low as 2.5–6 g/dL), hepatosplenomegaly, complications of iron overload, and skeletal deformities due to bone marrow expansion [123, 132]. Endocrinopathies, particularly hypogonadism, are frequent complications as the result of chronic anemia and iron overload [123]. Children with β -thalassemia major are at risk for parvovirus B19-induced aplastic crisis [124, 126].

β -Thalassemia intermedia represents patients with intermediate severity [135]. These patients usually have a compound heterozygous thalassemia consisting of more than one mutation.

The therapeutic approaches are based on the severity of the clinical manifestations and the thalassemia subtype. Patients with β -thalassemia minor usually do not need any specific treatment. Most patients with β -thalassemia intermedia need transfusion therapy, which can be delayed by splenectomy in certain patients [126]. Iron chelation therapy may be indicated [136]. Therapy for β -thalassemia major includes chronic hypertransfusion, splenectomy, iron

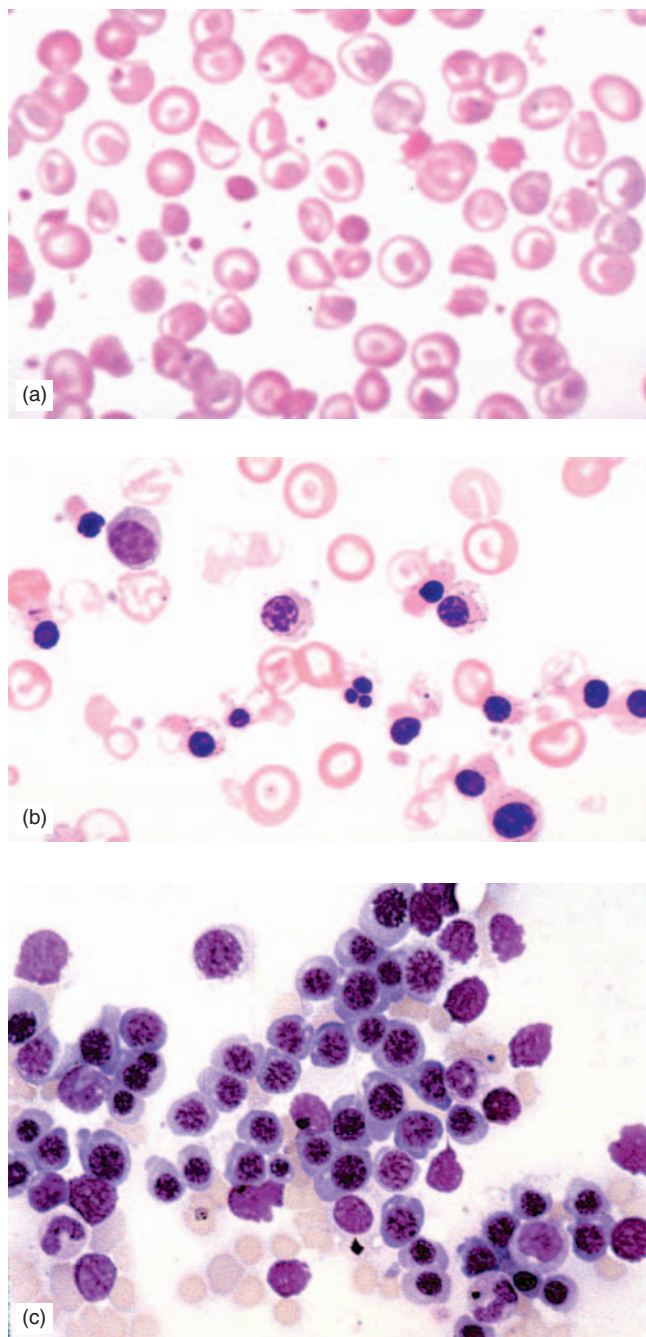


FIGURE 23.21 β -Thalassemia major. Blood smears showing target cells and abnormal nucleated red cells (a and b). Bone marrow smear shows erythroid hyperplasia (c).

chelation, hematopoietic stem cell transplantation, and molecular therapy [123, 137–140].

α -Thalassemia

α -Thalassemia results from the deletion of one or more α -globin genes (Figure 23.20) [141–143]. The single α -globin gene deletion appears to be the most common mutation worldwide [141–143]. The α -globin gene deletion, similar to other types of hemoglobinopathies, is believed to be protective against malaria infection [144, 145]. The non-deletion

mutations generally fall into three major categories: RNA processing defect, RNA translation defect, and post-translational instability [146, 147].

The deficiency in α -chain production results in the accumulation of excessive γ - and/or β -globin chains in α -thalassemia which may lead to the formation of unstable homotetramers β_4 (Hb H) and γ_4 (Hb Bart's) [148–150]. The severity of clinical manifestation and anemia in α -thalassemia correlates with several factors including the affected gene (α_1 or α_2), the total number of the affected α -globin genes, and the extent of functional loss resulting from specific mutations. For example, the α_2 -globin gene is expressed at 2.6-fold higher levels than the α_1 -globin gene and therefore plays a more significant clinical role [121].

Pathology and Laboratory Studies

Pathologic features are similar to β -thalassemia and correlate with the severity of the clinical manifestations. In general, symptomatic patients show a hypercellular bone marrow with marked erythroid hyperplasia and left shift. The polychromatophilic normoblasts are poorly hemoglobinized and show ragged cytoplasm. The iron stores are increased with abundant iron-laden macrophages.

The peripheral blood shows marked microcytic hypochromic anemia with low MCV and MCH and elevated RDW and reticulocyte count. Anisocytosis is prominent, and there is a variable degree of poikilocytosis, target cell formation, and basophilic stippling. Red cells may contain Hb H (tetramers of β -globin chains), which are precipitated when exposed to oxidants such as supravital stains, methylene blue and brilliant cresyl blue. Hb H precipitates are small, evenly dispersed deposits creating a golf ball appearance. Nucleated RBCs are often present. The WBC count may be elevated but the platelet count is within normal limits. The serum iron and ferritin levels are elevated. The transferrin saturation rate (ratio of serum iron to transferrin) is also high.

Hb electrophoresis, HPLC, and molecular genetic studies are used for the diagnosis of thalassemia syndromes and other hemoglobinopathies (see Figure 23.22) [130]. The number of deleted α -globin genes can be determined by Southern blot analysis or dosage-dependent quantitative PCR.

Clinical Features

There are four types of α -thalassemia: (1) a silent carrier with three functional α -genes, (2) α -thalassemia trait with two functional α -genes, (3) Hb H disease with only one functional α -gene, and (4) Hb Bart's (hydrops fetalis) or α_0 -thalassemia with no functional α -gene [121, 141, 146, 148].

The silent carrier form is prevalent in the Mediterranean basin, Middle East, India, Southeast Asia, and Indonesia and affects approximately 30% of Afro-Americans [121, 148, 151]. The affected individuals are asymptomatic and display unremarkable hematologic parameters except for a borderline low MCV and MCH. The Hb A2 concentration is normal. The diagnosis is usually made by molecular genetic studies.

Patients with α -thalassemia trait have no clinical symptoms but display mild anemia. The MCV and MCH are low and the Hb A2 concentration is normal. It affects approximately 3% of Afro-Americans [152].

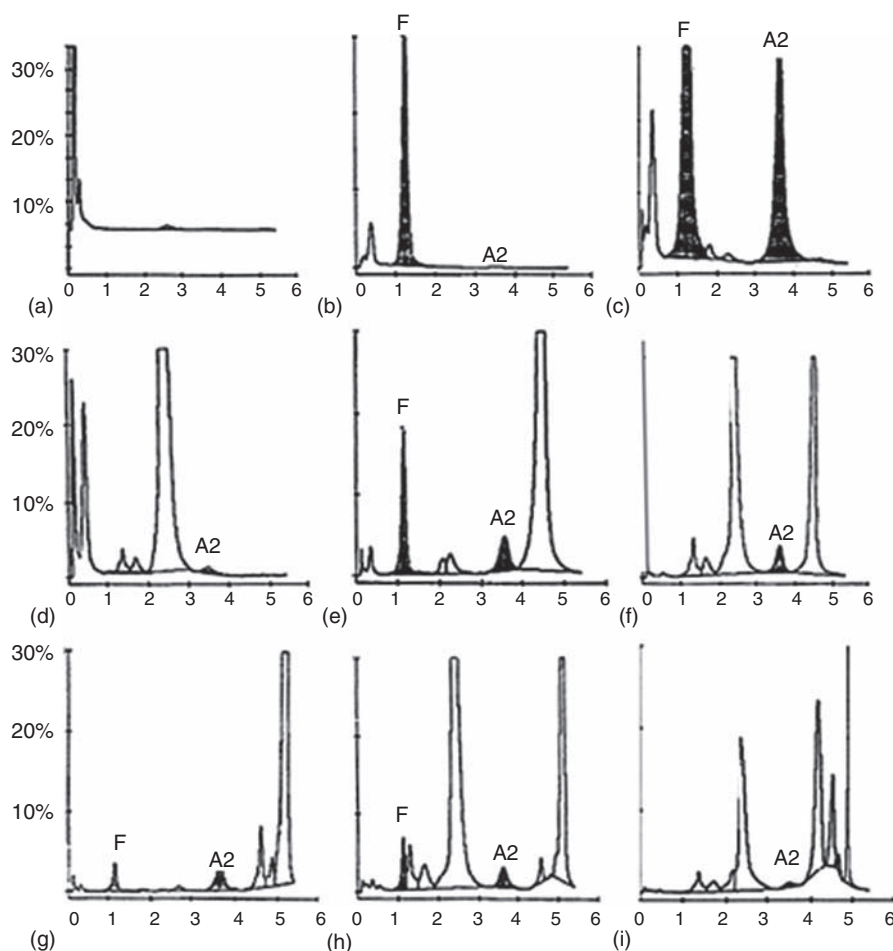


FIGURE 23.22 HPLC on the Bio-Rad Variant β Thal short program for Hb Bart's (a), β_0 thalassemia major (b), β_4 thalassemia homozygous E (c), Hb H (d), homozygous S (e), S trait (f), homozygous C (g), C trait (h), and Hb S-Hb G_{Philadelphia} (i). From Ref. [131] by permission.

The Hb H disease is associated with the precipitation of β -globin chain tetramers, leading to red cell membrane damage and hemolysis. Patients suffer from moderate to severe anemia and often demonstrate hepatosplenomegaly. Diagnosis is made by demonstration of Hb Bart's in 15–30% of the cord red cells at birth or by detection of Hb H (see Figure 23.8b) [148].

Hb Bart's leads to hydrops fetalis, the most severe form of α -thalassemia [148, 150]. Hb Bart's binds oxygen with high affinity and cannot release it to tissues. The affected fetuses have severe anemia leading to congestive heart failure with anasarca and capillary leak (hydrops) [150]. This condition is incompatible with extrauterine life.

The therapeutic approaches for α -thalassemias are similar to those discussed for β -thalassemias.

Other Thalassemia Variants

In addition to the classical hereditary α - and β -thalassemias, there are reports of acquired Hb H disease associated with myelodysplastic syndromes and myeloproliferative disorders [153, 154]. There are also variants of β -thalassemia associated with other structural abnormalities of β -globin chain such as Hb S, Hb C, and Hb E.

$\delta\beta$ -Thalassemia is the result of deletions of the δ - and β -globin genes or crossover between part of the δ locus on one chromosome and part of the β locus on the complementary

chromosome. The $\delta\beta$ -gene crossovers are apparently caused by misalignment of chromosome pairing during meiosis, resulting in a $\delta\beta$ -fusion gene. The gene product is an abnormal hemoglobin called *Hb Lepore* [155, 156].

Acquired α -thalassemia (Hb H disease) has been reported in myelodysplastic and myeloproliferative disorders. Two mechanisms have been proposed for this acquired process: (1) acquired deletion of the α -globin gene cluster limited to the abnormal hematopoietic clone and (2) inactivation of somatic mutations of the transacting chromatin-associated factor ATRX leading to downregulation of α -globin gene expression [153, 154].

Differential Diagnosis

The differential diagnosis of microcytic anemias, in addition to IDA and thalassemia syndromes, includes ACD, sideroblastic anemia, and anemias due to copper deficiency and zinc toxicity. The patient's clinical history and presentation help to distinguish these different categories. IDA is the only microcytic anemia with reduced serum ferritin and reduced or absence of the bone marrow iron stores (Table 23.9). The proportion of Hb A2 and fetal Hb is elevated, and Heinz bodies are present in β -thalassemias, and the presence of Hb H is the hallmark of α -thalassemia.

TABLE 23.9 Laboratory findings in microcytic anemias.*

	Serum Fe	TIBC*	% Saturation	Serum ferritin	Marrow Fe stain
Iron deficiency	↓	↑ (<16%)	↓	↓	Absent
Chronic disease	↓	↓	↓ or N	↑	↑
Sideroblastic	↑ or N	↑	↑	↑	↑ + RS**
Thalassemia	↑ or N	↑ or N	↑	↑	↑

*Total iron binding capacity (transferrin).

**Ringed sideroblasts.

SICKLE CELL DISEASE

Sickle cell (SC) disease consists of a family of hereditary hemoglobinopathies caused by mutations in the β -globin chain gene [157–159]. It includes Hb S (sickle β -globin), Hb C, and Hb E disorders. Hb D disease is the result of either β - or α -chain gene mutation. Sickle cell (SC) anemia is a homozygous state which represents the most severe form of the disease in this group, resulting from the inheritance of Hb S from both parents [160, 161].

Etiology and Pathogenesis

The Hb S mutation is a single base change in the DNA (GAG to GTG) in codon 6, which results in the substitution of the amino acid valine for glutamic acid at the sixth amino acid position in the β -globin chain. Deoxygenated Hb S molecules align in liquid crystals (tactoids) and distort the erythrocytes into rigid sickle shapes [159, 162, 163]. Reoxygenation disassembles the tactoids, and the erythrocytes become discoid and flexible again. Repeated sickling and unsickling is associated with red cell membrane changes such as changes in the membrane phospholipid composition and perturbation of the interaction between membrane phospholipids and cytoskeletal proteins. These changes eventually lead to the inability of the erythrocytes to switch from sickle shape to discoid shape [164, 165]. The irreversible SCs have a short intravascular life span and, because of their rigid shape, may block small vascular structures, leading to ischemia and endothelial cell damage [162, 166].

In Hb C, glutamic acid in the sixth position of the β -globin chain is replaced by lysine [167, 168]. The Hb C-containing erythrocytes are more rigid than normal red cells and may form rod-like crystals in hypoxic conditions. Erythrocyte damage and fragmentation may lead to the formation of microspherocytes.

Hb D involves either the β -globin chain or the α -globin chain. In the β -globin chain variant, glutamate is substituted for lysine at the 121th position, and in the α -globin variant, also known as Hb G_{Philadelphia}, asparagine is replaced by lysine at the 68th position [169, 170]. Several other variants of Hb D have been reported.

Hb E is the result of a β -chain mutation in which glutamine is substituted for lysine at the 26th position [171, 172].

Pathology and Laboratory Studies

Pathologic features correlate with the severity of anemia. In general, patients show a hypercellular bone marrow with marked erythroid hyperplasia and left shift. The extensive erythroid response and bone marrow overgrowth may lead to skeletal deformities [173]. Bone deformities may be secondary to bone infarcts, such as irregularities in the size of fingers and toes, partial vertebral collapse, or avascular necrosis of the pelvis. The femoral shafts, which in normal conditions are not actively involved in hematopoiesis, may become active. Extramedullary hematopoiesis may occur.

Blood examination in patients with homozygous SC anemia reveals anemia with a normal MCV, an Hb level ranging from 5 to 11 g/dL, and reticulocytosis (5–15%) (Figure 23.23). There is anisopoikilocytosis with the presence of SCs, target cells, and nucleated RBCs. Serum indirect bilirubin and lactate dehydrogenase levels are elevated, and serum haptoglobin and creatinine concentrations are low [174]. Hb C disease is often associated with the presence of rod-like crystals (Figure 23.24). Fetal Hb is slightly to moderately elevated.

Diagnosis of SC disease is established by the demonstration of Hb S on hemoglobin electrophoresis, HPLC, and by molecular genetic studies (Figure 23.25). In alkaline Hb electrophoresis, Hb S moves slower than Hb A and occupies a position between Hb A and Hb A₂. Hb C and Hb E move slower than Hb S, whereas Hb D has a mobility similar to that of Hb S. These variations are further delineated by an agar gel electrophoresis at an acid pH [175, 176]. A simple inexpensive test, SC test, has been traditionally used for the screening of patients suspicious of SC disease. This test is based on the principle that the reducing agents, such as sodium metabisulfite, are able to induce precipitation of Hb S in erythrocytes and cause sickle cell formation.

Molecular studies such as Southern blot analysis and polymerase chain reaction (PCR) have been used for the diagnosis of SC anemia and related disorders [176, 177]. For prenatal testing, fetal DNA samples are obtained from chorionic villi at 8–10 weeks gestation or from amniocentesis at 18 weeks. Although isolation of fetal SCs from maternal circulation has been reported, such approaches are not yet routinely utilized [178, 179].

Clinical Aspects

The prevalence of Hb S is greatest in Africa with a heterozygote frequency of about 20%. In Afro-Americans, SC

Dactylitis (acute pain in fingers and toes) before age 1, Hb concentration <7 g/dL, and leukocytosis in the absence of infections are considered predictors of an adverse outcome [187–190]. The estimated survival rate at 18 years in one large study was reported at about 88% [188, 189].

Other forms of SC disease and related disorders such as SC trait, Hb SC, Hb C, Hb D, Hb E, Hb S- β -thalassemia, and Hb C- β -thalassemia cause either no symptoms or mild symptoms with slight to moderate anemia.

The therapeutic approaches include treatment with hydroxyurea to increase Hb F levels, blood transfusion, iron chelation therapy, administration of antibiotics for prevention and treatment of the infections, and anticoagulation therapy to prevent or treat vaso-occlusive events [191–193]. Bone marrow transplantation may be effective in selected patients [194].

Differential Diagnosis

Family history, clinical manifestations, and evidence of Hb S establish the diagnosis. Bone marrow samples during SC crisis may be markedly hypoplastic or may show evidence of parvovirus B19 infection.

OTHER HEMOGLOBINOPATHIES

So far, over 800 different mutations have been reported in human Hb genes [195]. The majority of Hb mutations are clinically asymptomatic and have been discovered in conjunction with large population studies. The most prevalent mutations, thalassemia syndromes, and Hb S and related disorders were discussed earlier in this chapter. Unstable Hbs are infrequent Hb mutations that are briefly discussed in the following section.

Unstable Hemoglobins

Mutations of the globin chains may result in the formation of Hb molecules that are less soluble and have increased tendency of precipitation in the erythrocytes, leading to red cell membrane damage and hemolysis. The intracellular Hb precipitates are detected by supravital stains as dark globular aggregates called Heinz bodies. Over 250 unstable Hb molecules have been identified [196–198]. Patients with unstable Hb demonstrate a broad spectrum of clinical manifestations ranging from normal Hb levels to severe anemia. Jaundice and splenomegaly may be present. In some cases, high oxygen affinity may cause polycythemia. Bone marrow is usually hypercellular and shows erythroid preponderance. Anisopoikilocytosis and reticulocytosis are frequent peripheral blood morphologic findings. The most frequent type of unstable Hb in the West is Hb Köln which is characterized by mild anemia, reticulocytosis, splenomegaly, and pigmenturia [198, 199].

ERYTHROCYTE MEMBRANE SKELETON DEFECTS

A group of hereditary hemolytic anemias are caused by defects in erythrocyte membrane skeleton. These disorders are relatively common and are characterized by abnormal shape and decreased deformability of the red cells [200, 201].

Etiology and Pathogenesis

The red cell membrane consists of a lipid bilayer, integral membrane proteins, and a complex skeletal protein comprising α - and β -spectrin, ankyrin, protein 4.1, protein 4.2 (pallidin), and actin (Figure 23.26) [200, 202–204]. The membrane skeleton proteins interact with the integral membrane protein band 3 (anion exchanger, AE1) and glycophorin C to provide erythrocyte integrity and deformability. The α - and β -spectrin subunits bind side-to-side and form flexible rod-like heterodimers which self-associate head-to-head and make tetramers [200]. The spectrin tetramers are linked by ankyrin to protein 4.2 and protein band 3. Protein 4.1 attaches to the integral membrane glycoprotein C and interacts with β -spectrin at the actin-binding domain to increase the affinity of the spectrin–actin binding [200]. The spectrin tetramers at their tail ends interact with actin protofilaments, tropomyosin, tropomodulin, and adducin to form junctional complexes [200]. The genes of the major erythrocyte membrane proteins have been mapped and cloned, and structural and functional domains in each protein have been characterized [200, 203, 205]. Mutations of these genes are associated with erythrocyte membrane skeleton defects.

Defects in spectrin and other membrane-associated skeletal proteins lead to membrane lipid loss and surface area deficiency, alteration in cation content and membrane permeability, and decreased deformability of the erythrocytes. The affected red cells are not able to pass through the Billroth cords to the splenic sinuses and stagnate in an environment that has a lower pH and a decreased level of glucose. The erythrocytes are eventually destroyed or removed by the splenic macrophages [205–207].

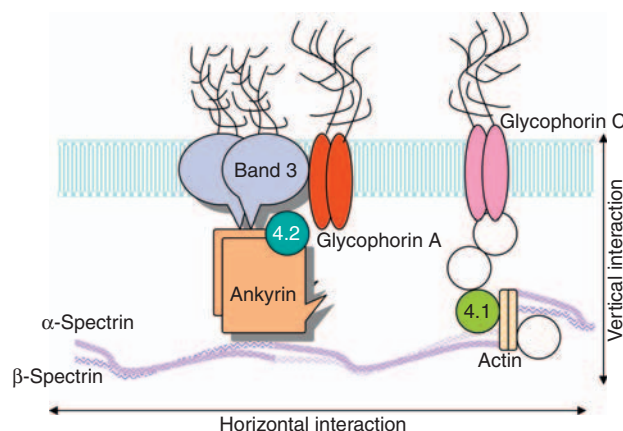


FIGURE 23.26 Schematic model of red cell membrane demonstrating vertical and horizontal interactions of various cytoskeletal components. Adapted from Ref. [200].

Clinical and Pathologic Features

Hereditary hemolytic anemias due to defective erythrocyte membrane cytoskeleton include clinicopathologic entities such as spherocytosis, elliptocytosis, acanthocytosis, and stomatocytosis.

Hereditary Spherocytosis

Hereditary spherocytosis (HS) is the most common cause of hemolytic anemia of non-immune nature characterized by the presence of numerous spherocytes in the peripheral blood (Figure 23.27a). The incidence of HS is significantly higher in northern European countries than in other parts of the world [207, 208]. The autosomal dominant form is the most frequent type accounting for approximately 75% of the cases. The disease is caused by defective erythrocyte membrane skeletal proteins in vertical interactions (see Figure 23.26), demonstrated by reduced β -spectrin (dominant) or α -spectrin (recessive) production, impaired binding to protein 4.1, and reduced or unstable ankyrin production (Table 23.10) [200, 207–209].

The clinical manifestation of HS may range from no symptoms to severe anemia. Most patients show mild to moderate anemia, mild jaundice, and a palpable spleen with a family history of anemia involving one or more siblings or parents. The peripheral blood smears reveal spherocytosis with a variable degree of reticulocytosis. MCHC and RDW are elevated [210]. Red blood cells show increased osmotic fragility. Occasionally, HS is complicated by aplastic crisis caused by parvovirus B19 infection [211, 212]. Patients with severe HS are treated with folic acid supplementation, blood transfusion, and splenomegaly.

Hereditary Elliptocytosis

Hereditary elliptocytosis (HE) represents a diverse group of hemolytic anemias characterized by the presence of numerous oval, elliptical, or elongated erythrocytes in the peripheral blood (Figure 23.27b). The prevalence is relatively low in the United States and Europe and high in the regions where malaria is endemic, such as Africa and Southeast Asia [213, 214]. The disorder is associated with defective erythrocyte membrane skeletal proteins in horizontal interactions, demonstrated by defective self-association of subunits or dimmer–dimmer association of spectrin and/or deficiencies of glycoprotein C, protein 4.1, or protein 4.2 (Table 23.10) [215–218]. HE has been divided into three major clinical groups: (1) common type, (2) spherocytic type, and (3) stomatocytic type.

Common HE is mostly reported as autosomal dominant disorder with no clinical symptoms or mild to moderate anemia. Jaundice, splenomegaly, and reticulocytosis may be present in some cases. An autosomal recessive variant of HE, *hereditary pyropoikilocytosis* (HPP), is characterized by severe hemolytic anemia, jaundice, splenomegaly, and marked spherocytosis and poikilocytosis [219]. HPP is found predominantly in the black population. Elliptocytosis may provide resistance against malaria [214, 220]. Similar to HS, patients with severe HE are treated with folic acid supplementation, blood transfusion, and splenomegaly.

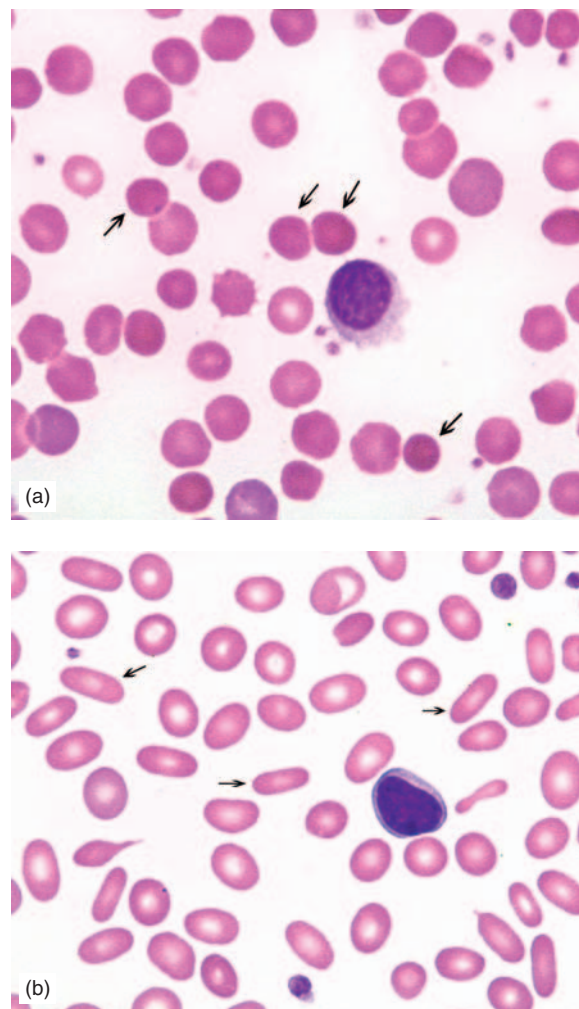


FIGURE 23.27 Blood smears demonstrate spherocytes (a) and elliptocytes (b).

Spherocytic HE (hereditary ovalocytosis) is associated with rounder erythrocytes that show increased osmotic fragility. It is an autosomal dominant disorder observed only in Caucasians. There is mild to moderate hemolysis with splenomegaly.

Stomatocytic HE has been reported in up to 30% of the population of Malayan aborigines in Southeast Asia and it is also known as Melanesian ovalocytosis [221]. It is characterized by spoon-shaped erythrocytes with absent or mild clinical manifestations.

Differential Diagnosis

The differential diagnosis of HS includes autoimmune hemolytic anemia (AIHA) and unstable hemoglobinopathies. The HS patients are negative for Coombs' test and lack Heinz bodies. Elliptocytosis and poikilocytosis have been associated with a garden variety of hematologic disorders such as IDA, thalassemia syndromes, megaloblastic anemia,

TABLE 23.10 Major abnormalities of skeletal proteins of the red blood cell membrane.*

Gene product	Chromosome	Phenotypic features	Defect
α-Spectrin	1q21	HS (recessive)	Spectrin 30–75% normal; abnormal proteolytic domain
		HE _c /HPP	Defective α-subunit self-association; abnormal proteolytic domain
β-Spectrin	14q22-q23.2	HS (dominant)	Spectrin 60–85% normal; impaired binding to protein 4.1
		HE _c	Diminished spectrin tetramer self-association; impaired binding to ankyrin
Ankyrin	8p11.2	HS	Spectrin 50% normal; unstable ankyrin
Protein 3	17q21-q22	Acanthocytosis	Reduced ankyrin binding
Protein 4.1	1p34-p36.2	HE _c	Partial or total 4.1 deficiency
Protein 4.2	15q15	HE _s	4.2 deficiency; decreased ankyrin stability
Glycophorin C	2q14-q21	HE _c	Weakened 4.1 association with membrane

*Adapted from Davies KA, Lux SE, McGuire M, Agre P. (1988). Clinical disorders of the erythrocyte membrane skeleton. *Hematol Pathol* **2**, 1–14.

HS: hereditary spherocytosis; HE_c: hereditary elliptocytosis, common type; HPP: hereditary pyropoikilocytosis; HE_s: hereditary elliptocytosis, stomatocytic type.

myelofibrosis, and myelodysplastic syndromes. Elliptocytes in these conditions, however, are generally below 60% of the red cells. Definitive diagnosis of HS or HE is established by identification of the underlying molecular defects of the erythrocyte membrane skeletal proteins.

Other Membrane-Associated Erythrocyte Abnormalities

Acanthocytosis

Acanthocytes, or spur cells, are red cells with multiple irregularly shaped and randomly distributed cytoplasmic projections (see Figure 23.3a). These abnormal erythrocytes are seen in a β-lipoproteinemia, amyotrophic chorea (chorea-acanthocytosis), McLeod syndrome (Kell antigen defects), cystic fibrosis, anorexia nervosa, severe liver disease, and hypothyroidism [222–226]. These disorders appear to cause overexpansion of the outer half of the membrane bilayer and formation of the irregular projections [225–227].

Acanthocytes should be distinguished from echinocytes (burr cells). Echinocytes are erythrocytes with evenly distributed pointed projections (see Figure 23.3b). Echinocytosis is observed in uremia, liver disease, hypomagnesemia, hypophosphatemia, post-chemotherapy, and in athletes after heavy physical exercise [228, 229]. Crenated red cells are cells with evenly distributed blunt projections, usually a common laboratory artifact caused by blood storage, contact with glass, or elevated pH (see Figure 23.3c).

Stomatocytosis

Stomatocytes are cup- or bowl-shaped erythrocytes which in blood smears appear as cells with a wide slit or stoma (mouth-like) area of central pallor (see Figure 23.2b). The stomatocyte shape is the result of the decreased ratio of the surface area to the volume in the erythrocytes. The increased red cell volume in almost all cases is due to increased permeability. Stomatocytes are trapped and consequently hemolyzed in the microvasculature of spleen and other organs. Stomatocytosis is either hereditary or acquired.

Hereditary stomatocytosis is an autosomal dominant genetic disorder leading to the increased permeability of the red cells to sodium [216, 230–232]. The increased permeability to sodium in some studies was associated with a deficiency of the erythrocyte membrane protein stomatin (band 7.2b) [232]. Hemolytic anemia with stomatocytosis (up to 40–60%), elevated reticulocyte count, elevated serum bilirubin levels, and reduced serum haptoglobin concentration in children or adolescents are characteristic features of hereditary stomatocytosis.

Acquired stomatocytosis is usually manifested in adults. It has been observed in alcoholism, chronic liver disease, malignancies, and cardiovascular disorders [233, 234].

HEMOLYTIC ANEMIA SECONDARY TO ERYTHROCYTE ENZYME DEFICIENCIES

Numerous enzymes are involved in the RBC metabolic activities such as Embden–Meyerhof glycolytic pathway, the Rapoport–Leubering shunt, and the pentose phosphate pathway. These metabolic activities maintain the structural and functional activities of the erythrocytes. Erythrocyte enzyme deficiencies are associated with a wide variety of clinical syndromes, some of which demonstrate hemolytic anemia of non-spherocytic type [235–239]. The most prominent erythrocyte enzyme deficiencies, glucose-6-phosphate dehydrogenase (G6PD) deficiency and pyruvate kinase (PK) deficiency, are briefly discussed here.

Glucose-6-Phosphate Dehydrogenase Deficiency

Glucose-6-phosphate dehydrogenase deficiency results from a wide variety of mutations in the *G6PD* gene located on X chromosome (Xq28) [235, 236, 240–242]. Over 350 mutations of *G6PD* gene have been defined. G6PD is an enzyme essential for basic cellular functions, including protection of red cell

proteins from oxidative damage [235, 236]. The G6PD activity is necessary for the generation of NADPH that is utilized for glutathione reduction [235, 236]. Reduced glutathione restores soluble Hb. The G6PD deficiency increases Hb vulnerability to oxidative damage, leading to Hb instability and precipitation as Heinz bodies [237, 243]. There are three clinical variants of G6PD deficiency associated with (1) acute intermittent hemolytic anemia, (2) chronic hemolysis, and (3) no obvious risk of hemolysis [235, 236]. The A-G6PD deficiency with acute intermittent hemolysis is the most common clinical presentation and is observed in Africans. The Mediterranean type of G6PD deficiency is more severe than the type A-G6PD deficiency. The G6PD deficiency affects about 10% of Afro-Americans and West Africans, 5–15% of Kurds, and 5–35% of Sardinians [240–242]. The distribution of populations with high frequency of G6PD deficiency geographically overlaps closely with the prevalence of malaria, suggesting that G6PD deficiency may play a protective role against malaria [244].

The vast majority of G6PD-deficient patients do not demonstrate hemolysis if they are not suffering from infection or are not exposed to oxidants [240, 245, 246]. Drugs, fava beans (particularly in patients with the Mediterranean type), and viral or bacterial infections are the most common inducers of hemolysis in the G6PD-deficient patients (Table 23.11) [235, 236].

During the hemolysis, red cells are normocytic normochromic or may show some degree of anisocytosis and poikilocytosis. Spherocytosis is not evident. Supravital stains may show Heinz bodies. The hemolytic episode stimulates erythropoiesis and leads to bone marrow erythroid hyperplasia and peripheral blood reticulocytosis. Similar to other hemolytic anemias, serum bilirubin level is elevated and serum haptoglobin concentration is reduced. G6PD activity is assessed by a fluorescent screening test or by quantitative spectrophotometric analysis. Alternatively, the actual gene mutation can be detected by DNA sequencing.

Patients with G6PD deficiency are instructed to avoid drugs and substances that may induce hemolysis. Patients who develop severe hemolysis may require blood transfusion. Folate supplementation is provided in those patients with chronic hemolysis [235, 236].

Pyruvate Kinase Deficiency

Pyruvate kinase (PK) deficiency is an autosomal recessive disorder with clinical manifestations of non-spherocytic hemolytic anemia in homozygotes or double heterozygotes [235, 236, 247–249]. PK is involved in the conversion of phosphoenolpyruvate to pyruvate and the generation of ATP in the Embden–Meyerhof pathway. Erythrocyte PK is synthesized under the control of the *PK-LR* gene located on chromosome 1 (1q21) [247, 250–253]. So far, over 150 *PK-LR* mutations associated with non-spherocytic hemolytic anemia have been reported [250]. PK deficiency is distributed worldwide, but it is more prevalent in people of northern Europe, China, and the Amish community in Pennsylvania [235, 236].

The severity of hemolysis in the PK-deficient patients is highly variable and may range from a mild fully compensated hemolysis with no anemia to chronic, severe,

TABLE 23.11 Examples of substances known to induce hemolysis in patients with glucose-6-phosphate dehydrogenase deficiency.

Acetanilid
Aspirin, high dose
Dimercaprol
Fava beans
Isobutyl nitrate
Methylene blue
Naphthalene
Nitrofurantoin (Furadantin)
Pamaquine
Pentaquine
Phenacetin
Phenylhydrazine
Primaquine
Sulfacetamide
Sulfonamide
Sulfapyridine
Toluidine blue
Vitamin K (water-soluble analogs)

life-threatening hemolytic anemia [235, 236, 247]. The red cell morphology is unremarkable or may show variable degrees of anisopoikilocytosis and reticulocytosis. Splenomegaly is a frequent finding. Severe cases may develop clinical complications, such as chronic jaundice, development of gallstones, folate deficiency, and transient aplastic anemia crisis, often due to parvovirus B19 infection. Quantitative PK enzyme assays establish the diagnosis. Molecular diagnostic methods, due to the large number of mutations and their low prevalence, are not in routine laboratory use [235, 236].

IMMUNE-MEDIATED HEMOLYTIC ANEMIA

Immune-mediated hemolytic anemia is the most common form of acquired hemolytic anemias. It is caused by a variety of antibodies against antigens expressed or attached to the patient’s red cell membrane. These include autoantibodies, antibodies against transfused red cells (ABO-incompatibility), maternal antibodies against fetal erythrocytes, and drug–antibody complexes [254, 255].

Autoimmune Hemolytic Anemia

Autoimmune hemolytic anemia (AIHA) is the most common type of acquired hemolytic anemias and is caused by the destruction of RBCs by autoantibodies directed against erythrocytes [254–256].

Etiology and Pathogenesis

Autoimmune hemolytic anemia is most often idiopathic. However, several etiologic factors have been proposed for

TABLE 23.12 Classification of autoimmune hemolytic anemia.

1. Serologic classification	
a.	Warm autoantibody (agglutinin): antibody with enhanced activity at 37°C
b.	Cold autoantibody (agglutinin): antibody with enhanced activity <37°C
c.	Mixed warm and cold autoantibodies
2. Classification based on etiology	
a.	Idiopathic
b.	In association with
i.	Lymphoproliferative disorders
ii.	Other malignancies (e.g. ovarian carcinoma)
iii.	Autoimmune disorders (e.g. SLE, ulcerative colitis)
iv.	Infections (e.g. mycoplasma, EBV)
v.	Drugs (e.g. α -methylidopa)

AIHA, such as altered self-antigen, abnormalities in antigen presentation, B-cell hyperactivity, and abnormalities in suppressor T-cell number and/or function [254–258]. One or a combination of these immunologic aberrations, in association with environmental and genetic factors (drugs, infections, malignancies), lead to an autoimmune hemolytic disorder.

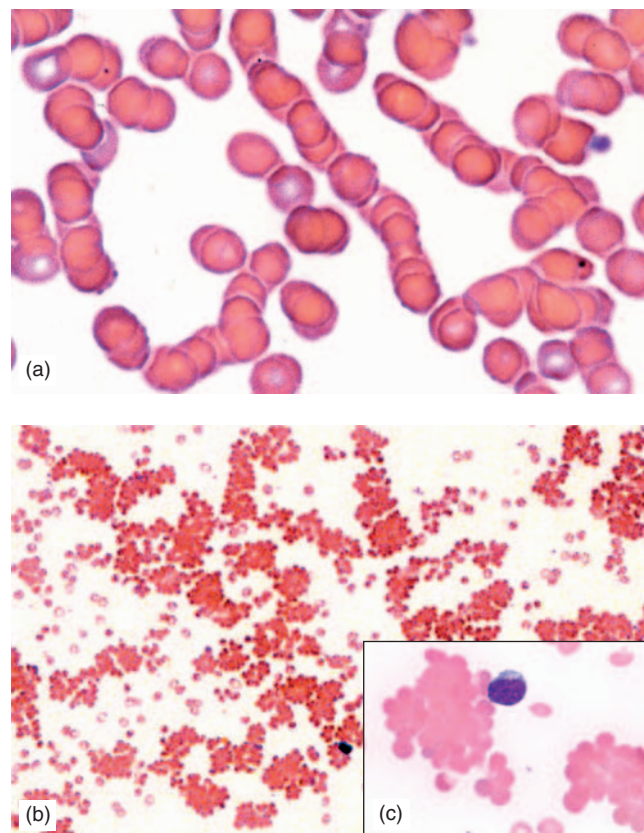
Autoimmune hemolytic anemia is divided into two major categories based on the nature of the antibodies (agglutinins): (1) warm-reacting antibodies and (2) cold-reacting antibodies (Table 23.12).

Warm-reacting antibodies (warm agglutinins) primarily represent IgG antibodies that react with red cell membrane proteins at body temperature. The IgG-coated red cells adhere to the Fc receptors of the tissue macrophages and are damaged either by erythrophagocytosis or by lysosomal enzymes released during antibody-dependent cellular cytotoxicity. Erythrophagocytosis is either complete or incomplete. Incomplete erythrophagocytosis leads to the formation of spherocytes. The red cell destruction occurs in tissues and therefore is called *extravascular hemolysis*. Approximately 2–3% of patients with severe AIHA demonstrate erythrocyte-bound IgA [259].

The efficacy of red cell–macrophage interactions depends on a number of factors such as the IgG subclass, the antibody concentration, and the concentration and subclass of Fc receptors on the macrophages [255, 257]. IgG1 and IgG3 are the most predominant Ig subtypes in macrophage-induced AIHA. Splenic macrophages are the most efficient effector cells and play a major role in the extravascular red cell destruction in autoimmune conditions [260].

Various conditions may initiate the autoantibody production and development of AIHA, such as viral infections (usually in children), collagen vascular diseases (especially systemic lupus erythematosus), lymphoid malignancies, prior allogeneic blood transfusion or stem cell transplantation, and certain drugs, particularly those used in the treatment of lymphoid malignancies (Table 23.12) [256, 261, 262].

Cold-reacting antibodies (cold agglutinins) in general consist of IgM antibodies that react with red cell membrane polysaccharides (such as I and i antigens)

**FIGURE 23.28** Blood smears demonstrate red cell rouleaux formation (a) and agglutinated red cells (b and c) for comparison.

at temperatures below body temperature [263]. Cold agglutinins are not very effective in extravascular hemolysis, because macrophages and other cytotoxic cells of the immune system do not carry IgM receptors. The red cell destruction is primarily due to the complement fixation and happens within the blood vessels (*intravascular hemolysis*) (Figure 23.28). The IgM autoantibodies at low temperature activate the complement cascade and bind the erythrocyte membrane, causing small holes in the red cell membrane and consequently hemolysis [264, 265].

Two major causes of cold agglutinin are infections and lymphoid neoplasms (Table 23.12). The most frequent infections associated with cold agglutinin production are *Mycoplasma pneumoniae* and EBV [266, 267]. Chronic lymphocytic leukemia, Waldenstrom macroglobulinemia, and high-grade B-cell lymphomas are among the neoplasms that are frequently associated with cold agglutinins [268, 269].

Clinical and Pathologic Features

Autoimmune hemolytic anemia with warm-reacting antibodies occurs at any age, but the majority of the patients are over age 40 with a peak incidence at around age 70. Clinical features are highly variable, ranging from a very mild chronic anemia with no clinical symptoms to a severe acute form with jaundice, splenomegaly, and hepatomegaly. Most cases have a mild clinical course.

Peripheral blood examination shows various degrees of anisopoikilocytosis, spherocytosis, reticulocytosis, and the presence of nucleated red cells. In severe cases, peripheral blood monocytes may occasionally show erythrophagocytosis. Granulocytosis and thrombocytosis may occur, and some severe cases may demonstrate a leukoerythroblastic blood picture. The bone marrow shows erythroid hyperplasia.

Demonstration of IgG and/or complement bound to the patient's erythrocytes is a diagnostic test for AIHA. The use of polyclonal antiglobulin reagent (Coombs' test), which contains antibodies against IgG and complement components, is a routine screening procedure. In the direct antiglobulin test (DAGT), or direct Coombs' test, the patient's red cells are examined for the presence of bound Ig or complement [256, 262]. In the indirect antiglobulin test, the patient's serum is screened for the presence of autoantibodies. The Coombs' test can be quantitated by methods such as enzyme-linked immunoabsorbent assay (ELISA) or other immunoassay techniques [262, 270]. Monospecific antisera are used for further characterization of the autoantibody and the nature of the hemolytic process.

Autoimmune hemolytic anemia with cold-reacting antibodies (cryopathic hemolytic syndromes) is caused by autoantibodies which have enhanced activities below 37°C and usually below 20°C. These syndromes account for about 30% of AIHA. Two major syndromes are recognized in this group: cold hemagglutinin disease (CHAD) and paroxysmal cold hemoglobinuria (PCH).

Cold hemagglutinin disease is characterized by a positive DAGT due to the presence of complement (C3d and C4d) and high-titer anti-erythrocyte antibodies with maximum erythrocyte agglutination at 0–15°C. In cold weather, cold-reacting antibodies bind to erythrocytes and mediate complement fixation with cooler peripheral circulation, leading to red cell aggregation and intravascular hemolysis. Intravascular hemolysis is associated with anemia, hemoglobinuria, and hemosiderinuria. Acrocyanosis and splenomegaly may be present. Occasionally, CHAD develops as complication of EBV or *Mycoplasma pneumonia*, lasting for 1–3 weeks [271]. In such cases, the cold-reacting antibodies are polyclonal IgM, and often demonstrate anti-I or anti-I activities. A chronic form of CHAD exists, which is associated with lymphoproliferative disorders and is characterized by the presence of monoclonal IgM kappa with anti-I specificity in the serum.

Paroxysmal cold hemoglobinuria is a rare form of AIHA which has been characterized by episodes of massive intravascular hemolysis and hemoglobinuria in children when exposed to cold. The disease appears to be secondary to a number of viral infections (such as measles, chickenpox, mumps, and influenza) or congenital and tertiary syphilis [263, 264, 272]. Clinical features include fever, muscle pains, headache, vomiting, diarrhea, urticaria, and acrocyanosis. The antibody responsible for the hemolysis is of the IgG class, usually with anti-P specificity, able to bind to complement components at low temperature.

Therapeutic approaches in AIHA include blood transfusion, administration of corticosteroids, splenectomy, and the use of cytotoxic drugs, singly or in combination based on the severity of the anemia and its duration. Plasma exchange or plasmapheresis has been used in some patients with warm-reacting antibodies.

Differential Diagnosis

Spherocytosis, reticulocytosis, and positive antiglobulin (Coombs') tests are characteristic laboratory features of AIHA. AIHA is distinguished from hereditary spherocytosis by lack of a family history and positive antiglobulin tests. Alloantibody hemolytic anemia, sometimes observed in recipients of organ transplants, may mimic AIHA. Patients with PNH, similar to patients with CHAD, demonstrate hemoglobinuria. However, in PNH patients, flow cytometric studies for CD55 and CD59 show reduced or lack of expression of these antigens in blood cells, as well as positive acidified serum test and sucrose hemolysis test, whereas all these studies are negative in CHAD.

OTHER IMMUNE-MEDIATED HEMOLYTIC ANEMIAS

Hemolytic Transfusion Reactions

Hemolytic transfusion reactions occur when the recipient's plasma contains antibody against the transfused red cells [273–276]. In rare occasions, it also happens when plasma with high titer of antibody is transfused to a patient whose erythrocytes carry the relevant antigen.

Transfusion reactions may take place during or immediately after transfusion or may be delayed 6–8 days after transfusion [273, 274, 276]. Immediate transfusion reactions are typically the result of ABO incompatibilities. Rapid intravascular hemolysis may lead to hemoglobinuria and jaundice, and may be complicated by disseminated intravascular coagulopathy (DIC) and renal failure [273]. Fever, chill, chest pain, dyspnea, hypotension, rigors, vomiting, and diarrhea are among the initial clinical features.

Delayed transfusion reactions occur in previously immunized patients. Rh incompatibility is a classical example of this kind of reaction [277, 278]. The red cell destruction in delayed reactions is predominantly extravascular with a positive DAGT. Common features are anemia, fever, jaundice, and spherocytosis.

Hemolytic Disease of the Newborn

Hemolytic disease of the newborn is the result of interaction of maternal IgG antibodies (crossed placenta) and incompatible fetal red cells in fetal circulation [279, 280]. ABO and Rh incompatibilities are the first and second most common causes, respectively. The ABO type is less severe and occurs predominantly in group A or B infants and group O mothers. Rh-related hemolysis is more severe and may lead to intrauterine death or hydrops fetalis [281].

Drug-Induced Immune Hemolytic Anemia

Certain drugs may initiate an immune-mediated red cell destruction [282–284]. Three possible mechanisms have

been suggested for drug-induced immune hemolysis [257, 283].

1. Drug (or one of its metabolites) acts as a hapten and binds to the red cell membrane to generate antibodies. The antibody-coated red cells are then subject to extravascular clearance by the phagocytic system (Figure 23.29a). Penicillin and other cephalosporins are examples.
2. A drug–antibody complex formed in plasma leads to complement activation at the erythrocyte surface and, consequently, red cell hemolysis (Figure 23.29b). Examples are quinine, phenacetin, *para*-aminosalicylic acid, and sulfonamides. This mechanism has been challenged [285].
3. Certain drugs, such as α -methyldopa, initiate the formation of autoantibodies against red cells (Figure 23.29c). Approximately 10–25% of patients on long-term α -methyldopa therapy develop IgG anti-red cell antibodies, though <1% may develop overt hemolytic anemia. Possible mechanisms include inhibition of suppressor T-cell activation by methyldopa or interaction of methyldopa metabolites with red cell peptides to form antigens [286, 287].

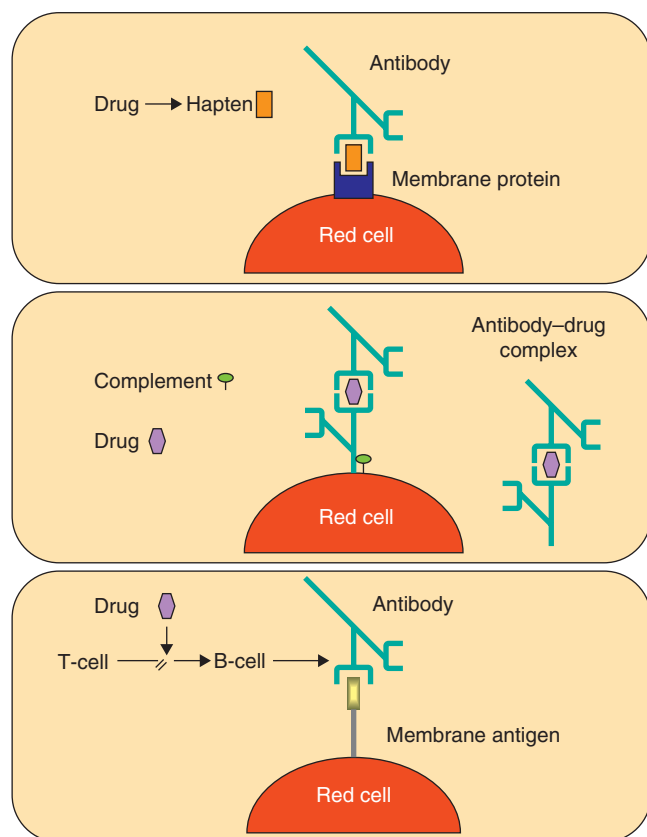


FIGURE 23.29 Schematic demonstration of possible mechanisms of drug-induced hemolytic anemia. Adapted from Naeim F. (1998). *Pathology of Bone Marrow*, 2nd ed. Williams & Wilkins, Baltimore.

ACQUIRED NON-IMMUNE HEMOLYTIC ANEMIAS

A wide variety of conditions may lead to an acquired non-immune hemolytic anemia. Examples include mechanical trauma and heat, drugs and other chemicals, infections, and hypersplenism.

Hemolysis Induced by Mechanical Trauma and Heat

Traumatic injury to the erythrocytes may cause red cell fragmentation and hemolysis. Erythrocyte fragmentation has been observed in patients with cardiac valve prosthesis, thrombotic thrombocytopenic purpura, malignant hypertension, generalized vasculitis, and carcinomatosis (Figures 23.30 and 23.31) [288–290]. Traumatic hemolysis and hemoglobinuria have also been reported soon after walking or running long distances, bongo-drumming, or karate exercise. Thermal damage is often associated with spherocytosis and generation of microvesicles [289].

Hemolysis Caused by Drugs and Other Chemicals

Drug-induced hemolysis unrelated to enzyme deficiencies is reported in various conditions such as exposure to arsenic hydride or nitrobenzene derivatives, copper, certain nitrites, and naphthalene [284, 291, 292].

Acute alcoholism may be associated with stomatocytosis and a transient hemolytic episode. Patients with alcohol-induced fatty liver may develop a syndrome (Zeive syndrome) characterized by episodes of hypercholesterolemia, hypertriglyceridemia, and hemolysis [293]. Venoms of certain species of snakes, bees, spiders, and wasps may cause hemolysis.

Hemolysis Caused by Infections

As discussed earlier, infection may initiate or provoke hemolytic episodes in patients suffering from red cell enzyme deficiencies or hemoglobinopathies, presumably due to the generation of oxidant substances. Certain microorganisms may damage erythrocyte membrane and cause hemolysis by the release of substances such as phospholipases (*Clostridium perfringens*) and hemolysins (*Streptococcus pyogenes*). Activation of macrophages in some viral or bacterial infections may lead to extensive erythrophagocytosis and anemia [294]. Malaria, particularly *Plasmodium falciparum*, may cause hemolysis.

Hypersplenism

One of the main functional roles of the spleen is to serve as a filter by removing foreign materials, cell debris, and defective blood cells [295]. This is accomplished by the passage

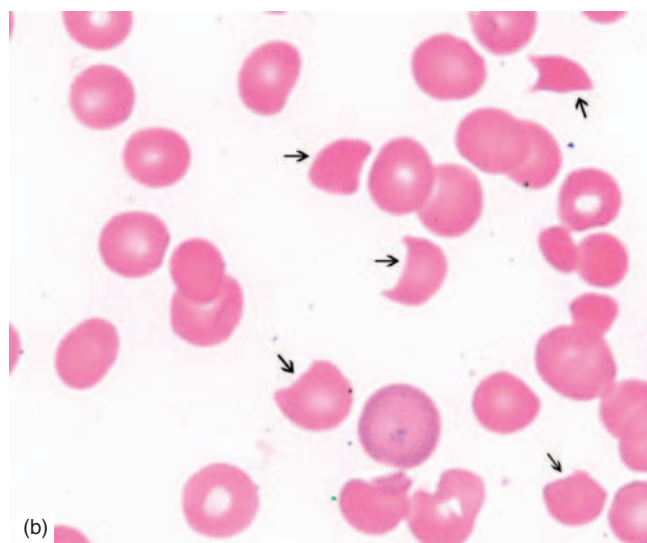
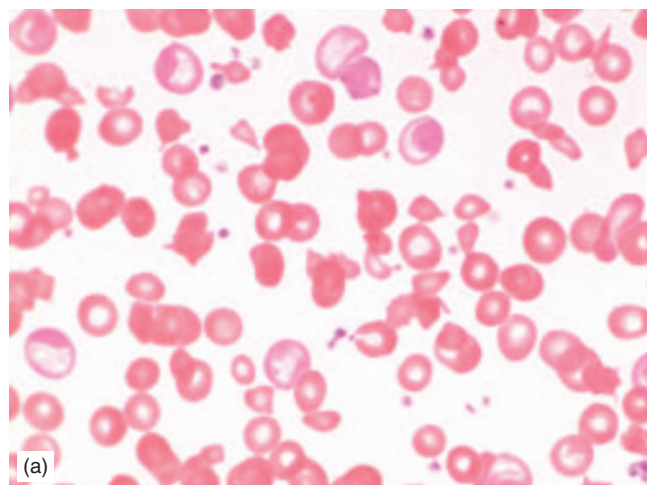


FIGURE 23.30 Microangiopathic changes. Blood smears demonstrate numerous schistocytes (a) and several helmet cells (b, arrows).

of a proportion of splenic blood supply through the non-endothelialized, macrophage-containing spaces present in the red pulp, functioning as a filter bed. In splenomegaly, the proportion of the blood channeled through the red pulp and the filter bed increases, causing an inappropriate sequestration of both normal and abnormal blood cells, particularly red cells, because of their limited, self-sufficient metabolic resources [296, 297]. The increase in splenic sequestration is more pronounced when the splenomegaly is caused by congestion than by an infiltrative process.

OTHER TYPES OF ANEMIA

Anemia of Chronic Disease

Anemia of chronic disease (ACD) is an anemia associated with chronic infections, chronic inflammations, and malignancies,

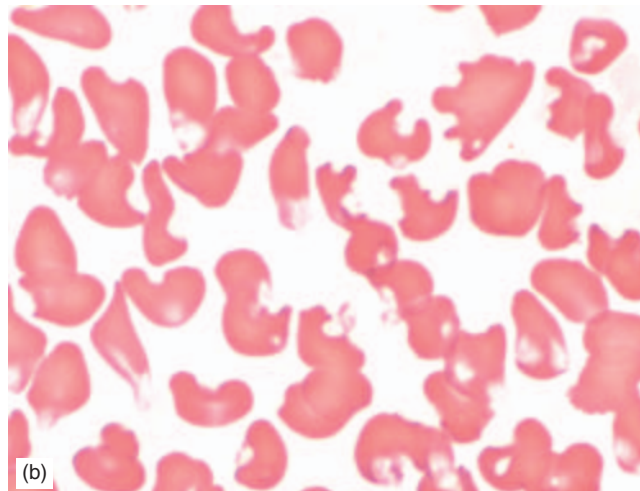
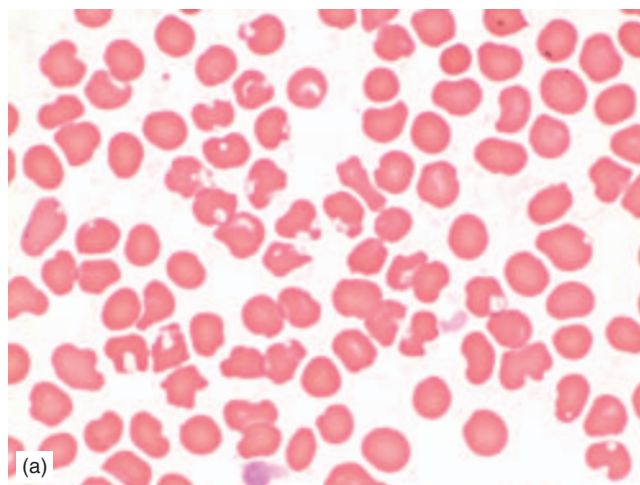


FIGURE 23.31 Blood smear shows schistocytes and punched out red cells in a patient with severe burn: (a) low power and (b) high power.

as well as a number of other conditions such as severe trauma, cardiovascular disorders, and diabetes mellitus [298–301].

Hepcidin appears to play an important role in iron metabolism and pathogenesis of ACD [302]. Hepcidin is an acute phase protein, a regulator of iron absorption in the small intestine and iron release from macrophages [303]. Increased hepcidin production has been observed in patients with ACD [304]. Recent studies suggest that IL-6 induces production of hepcidin [303].

ACD is characterized by a mild to moderate anemia, reduced absolute reticulocyte count, modest shortening of the erythrocyte life span, a low serum iron-binding capacity, an increased bone marrow iron store, and relatively ineffective erythropoiesis. Anemia is usually normocytic and normochromic, but it may be microcytic and hypochromic. Elevated levels of serum IL-6, fibrinogen, and C-reactive protein, as well as increased erythrocyte sedimentation rates, are frequently noted [305, 306].

An acute variant of ACD has been reported in patients with major trauma, myocardial infarction, and sepsis [300, 307].

The differential diagnosis includes microcytic and refractory anemias (see Table 23.9). ACD, unlike IDA, shows reduced serum TIBC and increased bone marrow iron stores. ACD is distinguished from refractory and sideroblastic anemias by reduced serum TIBC, lack of significant dysplastic changes, and absent ringed sideroblasts.

Deficiency Anemias Other than Vitamin B₁₂ and Folate

Copper Deficiency

Anemia of copper deficiency has been described in malnourished children and in patients receiving parenteral alimentation [308, 309]. Excessive and chronic administration of zinc may also cause copper deficiency. Copper deficiency is associated with microcytic anemia and neutropenia. The erythroid precursors in the bone marrow are often vacuolated.

Vitamin Deficiencies

Vitamin A and Vitamin B₆ deficiencies may lead to microcytic, hypochromic anemia. Vitamin B₂ (riboflavin) may cause pure red cell aplasia [310]. Anemia associated with vitamin C deficiency is usually normochromic and normocytic. Vitamin E deficiency, a common deficiency in patients with cystic fibrosis, is usually associated with abnormal red cell morphology and hemolysis [311].

Anemia of Chronic Renal Failure

A complex process is involved in the anemia of chronic renal failure consisting of a decline in erythropoietin production, the suppressive effects of uremia on erythropoiesis, and plasma inhibitors of heme synthesis [312, 313]. Anemia is usually normochromic and normocytic with a normal or reduced reticulocyte counts. Echinocytosis is often present. The bone marrow is normo- to hypocellular and may show severe erythroid hypoplasia.

Anemia Associated with Marrow Infiltration (Myelophthistic Anemia)

Metastatic carcinomas, particularly carcinoma of the lung, breast, and prostate, are the most common causes of bone marrow infiltration [314, 315]. Other causes of bone marrow infiltration are hematopoietic malignancies, marrow fibrosis, lysosomal storage diseases, and inflammatory processes such as granulomas. The infiltrative process disrupts the bone marrow's microenvironment and reduces hematopoietic activities. Myelophthistic anemia is often associated with anisocytosis and poikilocytosis, presence of teardrop red cells and leukoerythroblastosis [316]. Bone marrow biopsy sections are the most reliable source for the diagnosis of marrow infiltration.

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Disorders of Megakaryocytes and Platelets

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and
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MEGAKARYOCYTIC HYPOPLASIA

Megakaryocytic hypoplasia is one of the features of congenital or acquired aplastic anemia. A lack or markedly reduced production of megakaryocytes without bone marrow aplasia is rare and has been associated with prolonged administration of prednisone, estrogens, interferon, and chlorothiazide [1–3]. Chronic alcoholism and certain infectious diseases, such as measles, varicella, infectious mononucleosis, and cytomegalovirus, have been associated with megakaryocytic hypoplasia [2–4]. Amegakaryocytosis has also been reported in patients with thymic aplasia [5].

Congenital Megakaryocytic Hypoplasias

Congenital megakaryocytic hypoplasias are rare conditions characterized by thrombocytopenic purpura [6–10]. Two major subtypes have been described: (1) congenital amegakaryocytic thrombocytopenia (CAMT) and (2) congenital thrombocytopenia with absent radii (TAR).

CAMT is associated with an impaired response to thrombopoietin (TPO) due to the presence of mutations in the TPO receptor, c-Mpl [9, 10]. Serum TPO levels are elevated. Thrombocytopenia may be persistent with fast progression into pancytopenia (CAMT I) or transient and less severe CAMT II [10].

The TAR syndrome is associated with bilateral aplasia of the radii, cardiac and/or renal malformations, and dysmegakaryocytopoiesis characterized by cells blocked at early stages of megakaryocytic differentiation [7, 8]. Thrombocytopenia is usually severe and is sometimes associated with a transient leukemoid

reaction. Platelets may demonstrate abnormal function [11]. Bone marrow examination reveals a marked decrease in the number of megakaryocytes (Figure 24.1). TAR syndrome platelets demonstrate impaired Mpl expression compared to platelets from adult controls (Figure 24.2).

MEGAKARYOCYTOSIS

Megakaryocytosis and thrombocytosis are associated with acute infections, iron deficiency, diabetes mellitus, and postsplenectomy status [3, 11–13]. Postsplenectomy thrombocytosis is typically a transient event. Peripheral destruction of platelets in conditions such as autoimmune-associated thrombocytopenic purpura and drug-induced thrombocytopenia leads to megakaryocytosis secondary to bone marrow compensation.

Bone marrow metastasis is a frequent cause of reactive megakaryocytosis due to either the release of TPO-like substances from tumor cells or the increased expression of extracellular matrix (ECM) proteins such as tenascin [14, 15].

Megakaryocytosis is associated with a garden variety of primary bone marrow disorders such as myeloproliferative disorders, myelodysplastic syndromes, and acute megakaryoblastic leukemia (Figures 24.3 and 24.4). Unlike reactive megakaryocytosis, these conditions are often associated with significant dysplastic changes and arrangement of megakaryocytes in clusters or sheets.

Hereditary thrombocythaemia (HT) is an inherited autosomal dominant disorder caused by mutations within the genes encoding TPO or c-Mpl (TPO receptor) genes [16, 17].

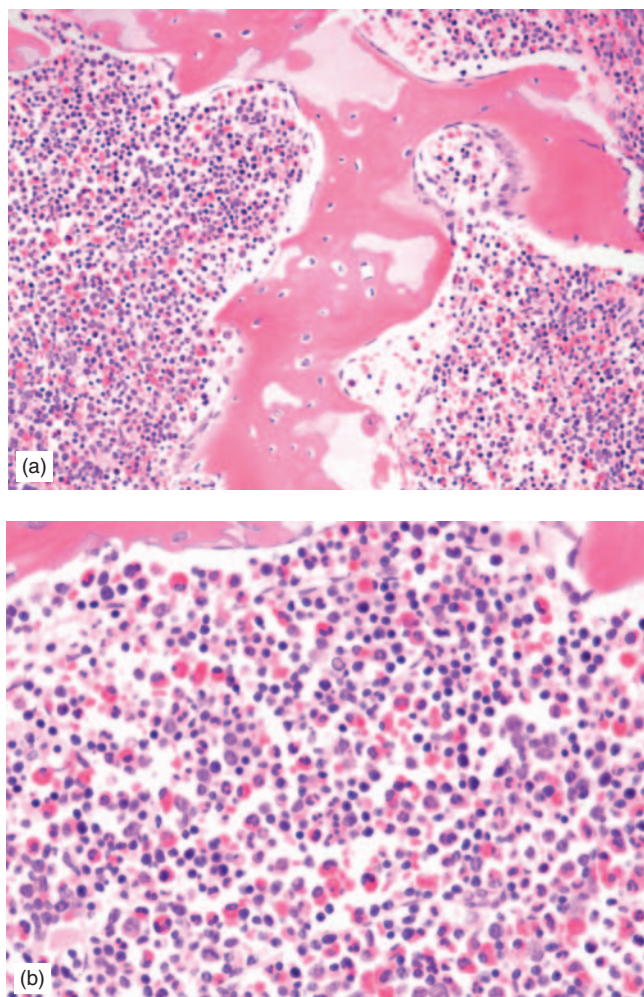


FIGURE 24.1 Bone marrow biopsy section of a child with TAR syndrome demonstrating markedly decreased megakaryocytes (a and b).

THROMBOCYTOPENIA

Thrombocytopenia is due to either impaired platelet production or increased platelet destruction. Impaired platelet production is caused by congenital and acquired aplastic anemias, paroxysmal nocturnal hemoglobinuria, congenital amegakaryocytosis, bone marrow metastasis, myelofibrosis, drugs, radiation, certain infections, and malnutrition. Increased platelet destruction may occur by two major mechanisms: (1) immunologic and (2) nonimmunologic.

Autoimmune Thrombocytopenic Purpura

Etiology and Pathogenesis

Autoimmune thrombocytopenic purpura (AITP), also known as idiopathic thrombocytopenic purpura (ITP), is an antibody-mediated thrombocytopenia [18–23]. In a vast majority of these cases, antiplatelet antibodies are against platelet membrane glycoproteins. Glycoprotein

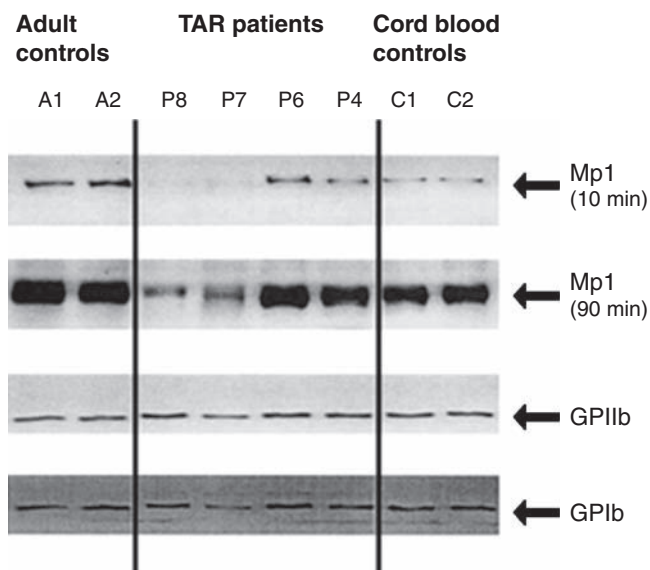


FIGURE 24.2 Western blot analysis of Mpl, GPIIb, and GPIb expression in platelets from normal adult controls, newborns, and TAR syndrome patients. Platelet lysates (20 µg) were separated by SDS-PAGE and probed with polyclonal antibodies against Mpl, GPIIb, and GPIbα. From Ref. [8] by permission.

(GP) IIb-IIIa (CD41/CD61) is the most frequent target [19, 21–24]. Autoantibodies against GPIIb-IX (CD42a) and GPV (CD42d) have also been reported [19, 25]. The autoantibodies are typically of the IgG class and can cross placenta, causing fetal thrombocytopenia in pregnant patients. A T-cell-mediated cytotoxicity has been suggested as an alternative mechanism in patients who do not demonstrate detectable autoantibodies [22, 26]. Molecular parody between GPIIb-IIIa and HIV proteins may play an important role in the pathogenesis of thrombocytopenia in patients with AIDS [22].

AITP is often associated with other autoimmune disorders such as systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, Hashimoto thyroiditis, ulcerative colitis, Crohn disease, biliary cirrhosis, myasthenia gravis, and pernicious anemia [27–31]. Other diseases such as sarcoidosis, lymphoproliferative disorders, Gaucher disease, IgA deficiency, and panhypogammaglobulinemia may demonstrate AITP [32–35].

Clinicopathologic Features

AITP has two clinical presentations: acute and chronic. Acute AITP is predominantly observed in children between the ages of 2 and 9 years. It is one of the most common causes of thrombocytopenia in children and is often preceded by a history of viral infection such as chickenpox, rubella, or rubeola [18, 19, 21]. Vaccination for measles, mumps, or chickenpox viruses may occasionally lead to AITP. It is characterized by a severe onset of thrombocytopenia leading to petechiae, purpura, as well as gastrointestinal and/or genitourinary tract hemorrhages. Intracranial bleeding is

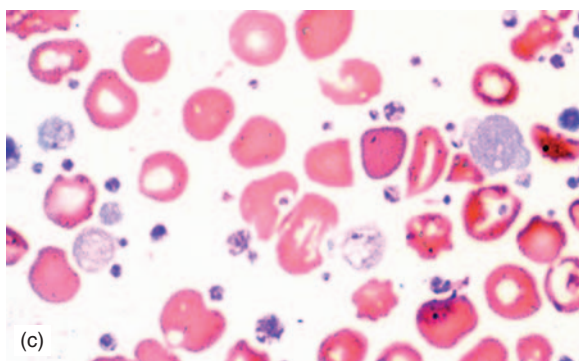
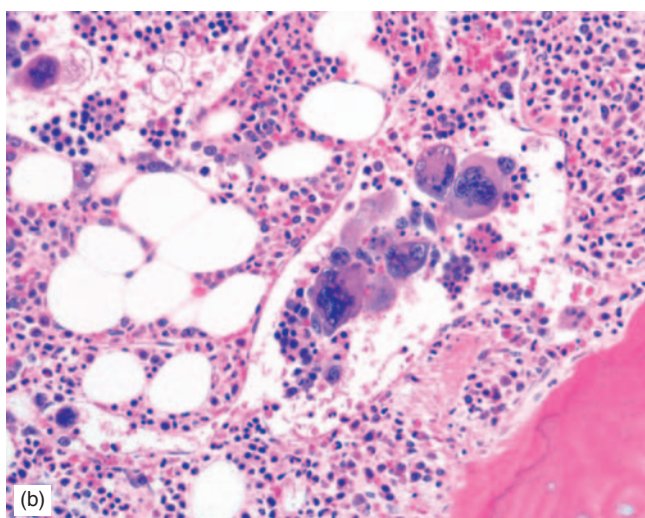
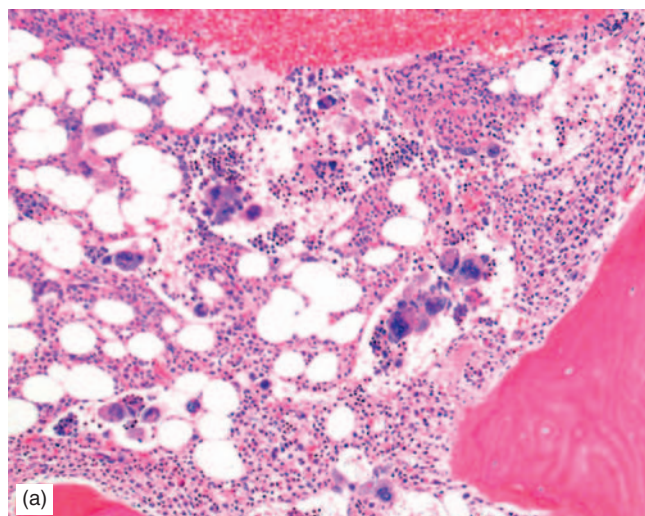


FIGURE 24.3 Bone marrow biopsy section from a patient in early stage of idiopathic myelofibrosis demonstrating megakaryocytosis. Aggregates of megakaryocytes are present in dilated sinuses: (a) low power and (b) high power views. Blood smear (c) shows thrombocytosis with several giant platelets.

rare. In approximately 80% of affected children, the thrombocytopenia resolves spontaneously within 6–12 months.

Chronic AITP occurs in adults, usually between 20 and 50 years of age [18, 19, 22]. The female:male ratio is

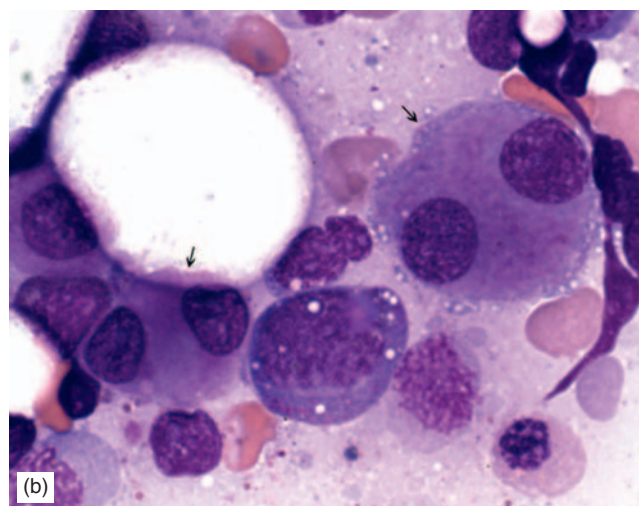
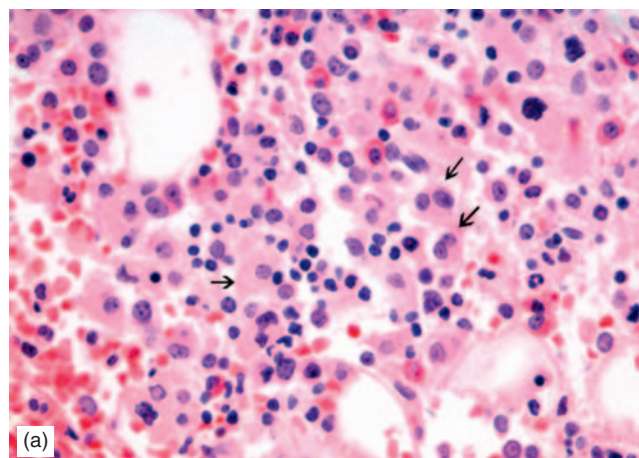


FIGURE 24.4 Biopsy section (a) of a patient with myelodysplastic syndrome showing numerous micromegakaryocytes. Bone marrow smear (b) demonstrates a binucleated micromegakaryocyte.

about 3:1. Overall, thrombocytopenia is less severe, and the chance of spontaneous remission is less frequent than the acute form of AITP. Clinical manifestations may vary from scattered petechiae to purpuric and ecchymotic lesions, epistaxis, and even intracranial bleeding.

Examination of the peripheral blood reveals thrombocytopenia with marked variation in the shape and size of the platelets. The platelet count may reach $<30,000/\mu\text{L}$ with prolongation of bleeding time. Rarely patients may also demonstrate an autoimmune hemolytic anemia (Evans syndrome). Iron deficiency anemia may occur due to chronic or excessive bleeding.

The clinical use of antiplatelet antibody studies for the diagnosis of AITP is controversial [22, 36]. In several studies, the rate of positive results for antiplatelet antibody tests ranged from 49% to 66% in patients with AITP and 7% to 28% in patients with nonimmune thrombocytopenia (e.g. congenital thrombocytopenia, myelodysplastic syndrome) [22, 37, 38].

Bone marrow examination reveals megakaryocytosis, diffusely dispersed between other hematopoietic cells. Micromegakaryocytes and giant megakaryocytes are often

present. Some megakaryocytes may show more basophilic and less granular cytoplasm with no platelet budding [3].

AITP in children is usually self-limiting with spontaneous remission. In adults, <10% of the patients may achieve spontaneous remission. In life-threatening forms, treatment includes platelet transfusion and intravenous immunoglobulin. Patients with mild to moderate thrombocytopenia are treated with corticosteroids, followed by splenectomy in nonresponders or in patients who relapse [21, 22, 39–41].

Differential Diagnosis

Differential diagnosis includes all conditions associated with thrombocytopenia, such as congenital thrombocytopenia, infections, primary bone marrow disorders, drugs, and disseminated intravascular coagulation (DIC). EDTA-dependent agglutinins may also cause platelet clumping and platelet satellitism around leukocytes and therefore a reduction in the platelet count (pseudo-thrombocytopenia).

Drug-Induced Immune Thrombocytopenia

The most common cause of drug-induced thrombocytopenia is immune mediated with two major proposed mechanisms:

1. The drug or its metabolites bind to a plasma protein, and antibodies are generated against the drug–protein complexes.
2. The drug binds to one or more components of the platelet membrane and induces a structural change. This drug–platelet complex provokes antibody production.

Platelet membrane glycoproteins such as GPIIb-IX (CD42a) and GPIIb-IIIa (CD41/CD61) are the primary targets [42–46]. Quinine, quinidine, sulfonamides, organic arsenicals, sulfisoxazole, ranitidine, rifampin, alpha methyl-dopa, para-aminosalicylate, heparin, and gold salts are among the drugs that have caused immune-mediated thrombocytopenia [42–48].

Heparin-induced thrombocytopenia (HIT) accounts for 10–20% of patients receiving unfractionated heparin [49–51]. HIT is frequently associated with thromboembolism. Two different mechanisms have been proposed for heparin-associated thrombocytopenia: (1) a direct heparin–platelet interaction leading to platelet activation, aggregation, and clearance (HIT type I) and (2) an immune-mediated thrombocytopenia (HIT type II) [49, 50]. Approximately 2% of HIT is immune mediated. Antibodies raised against heparin–platelet surface membrane complexes trigger platelet activation and aggregation and eventually immune clearance of the affected platelets. Platelet aggregates may cause emboli in both the arterial and the venous circulation.

Neonatal Immune-Mediated Thrombocytopenia

Neonatal immune-mediated thrombocytopenia is caused when maternal antiplatelet antibodies cross the placenta and bind to fetal platelets [52, 53]. Antiplatelet antibody production in the mother is due to either feto-maternal

incompatibility in platelet antigens (neonatal alloimmune thrombocytopenia) or maternal AITP. Alloimmune thrombocytopenia is caused by maternal IgG antibodies raised against fetal platelets. The most common cause of neonatal alloimmune thrombocytopenia is feto-maternal incompatibility in human platelet alloantigens (HPA)-1a, accounting for 50–90% of the cases [54, 55]. The risk of maternal sensitization correlates with the maternal human leukocyte antigen (HLA) type and appears to be confined to HLA-B8 and HLA-DR3 mothers. Affected children present with scattered or generalized petechial and/or purpuric hemorrhages at birth or soon after delivery. Intracranial hemorrhage may occur in up to 25% of the affected neonates [56]. Thrombocytopenia may persist for 2–3 weeks with a platelet count of around 30,000/ μ L or lower.

Thrombocytopenia associated with maternal AITP is observed in up to 50% of neonates born to mothers with AITP. The maternal IgG autoantibody crosses the placenta and binds to fetal platelets. Thrombocytopenia may last for 1–6 months. The incidence of spontaneous abortion is increased in mothers with AITP.

Posttransfusion Purpura

Posttransfusion purpura is caused by platelet alloantigenic incompatibilities between the recipient and the donor [57, 58]. It is usually manifested 7–10 days after blood or platelet transfusion. Alloantibodies are usually against HPA-1a. It is most commonly observed in recipients with specific HLA class II phenotypes, particularly in those who are HLA-DR3-positive [59, 60].

Thrombotic Thrombocytopenic Purpura/Hemolytic Uremic Syndrome

Thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) are overlapping acute syndromes with abnormalities in multiple organ systems [61–65]. Neurologic abnormalities are the dominant clinical features in TTP, and acute renal failure is the main clinical manifestation in HUS. The peak incidence of TTP is in the third decade, while HUS is observed primarily in infancy and early childhood.

Several etiologic factors have been postulated, which are primarily related to endothelial cell damage. Abnormalities of endothelial cell function, such as defects in prostacyclin metabolism and deficiency of plasminogen activator, have been demonstrated in some patients with TTP [66–69]. Recent studies suggest that TTP is caused most commonly by an autoimmune-mediated deficiency of the circulating von Willebrand factor (VWF) cleaving metalloprotease, ADAMTS13 [70]. There is a close association between TTP and collagen vascular disorders such as systemic lupus erythematosus, rheumatoid arthritis, Sjogren syndrome, and polyarteritis nodosa [71–73]. Certain viruses and bacteria such as HIV, coxsackie A and B, *Mycoplasma pneumoniae*, and *Legionella pneumophila* have been associated with TTP.

HUS is strongly associated with *Escherichia coli* O157:H7, which produces the Shiga-like toxins Stx-1 and Stx-2.

These toxins damage endothelial cells and cause increased release of inflammatory mediators by these cells [64, 74]. Other microorganisms reported in association with HUS are *Pneumococcus pneumoniae*, *Yersinia pseudotuberculosis*, *Salmonella typhi*, varicella, coxsackie enteroviruses, and ECHO virus [75–81].

Reports of the hereditary forms of TTP/HUS suggest a genetic predisposition in pathogenesis of this disorder in some patients [82, 83].

The characteristic clinicopathologic features of TTP/HUS are fluctuating neurologic symptoms (more in TTP), acute renal failure, and diarrhea (more in HUS), fever, microangiopathic hemolytic anemia, and thrombocytopenia with purpura, but usually not severe bleeding [62, 63, 84, 85].

Blood smears demonstrate moderate to severe anemia with anisopoikilocytosis, schistocytosis, and reticulocytosis. Nucleated red cells are often present. Thrombocytopenia is marked and in some patients it is $<20,000/\mu\text{L}$. The most prominent histologic finding is disseminated platelet/fibrin thrombi in the capillaries and arterioles of various tissues such as brain, kidney, pancreas, adrenal glands, and heart (Figure 24.5). Microthrombi are rarely detected in bone marrow biopsy sections.

Plasma exchange is the treatment of choice for all but minimally affected patients. Other therapeutic approaches include administration of corticosteroids, intravenous immunoglobulin, and antiplatelet aggregation agents [86].

Differential diagnosis includes conditions that are associated with hemolysis and thrombocytopenia, such as Evans syndrome (autoimmune hemolytic anemia and thrombocytopenia), DIC, systemic lupus erythematosus, microangiopathic hemolytic anemia in patients with disseminated cancers, and paroxysmal nocturnal hemoglobinuria.

Other Conditions Associated with Thrombocytopenia

A wide spectrum of clinical conditions are associated with thrombocytopenia, such as infections, glomerulonephritis, renal transplant rejection, renal vein thrombosis, DIC, giant cavernous hemangioma, burns, snake bites, aortic valvular disease, and primary pulmonary hypertension. Certain drugs such as hematin, protamine sulfate, bleomycin, and heparin can have direct damaging effects on platelets and cause thrombocytopenia. Administration of antithymocyte globulin may cause thrombocytopenia possibly due to the formation of immune complexes. Splenomegaly may cause thrombocytopenia due to sequestration of the platelets.

QUALITATIVE DISORDERS OF PLATELETS AND MEGAKARYOCYTES

Introduction

Analogous to the quantitative disorders just discussed, qualitative defects of platelet function result in a hemorrhagic

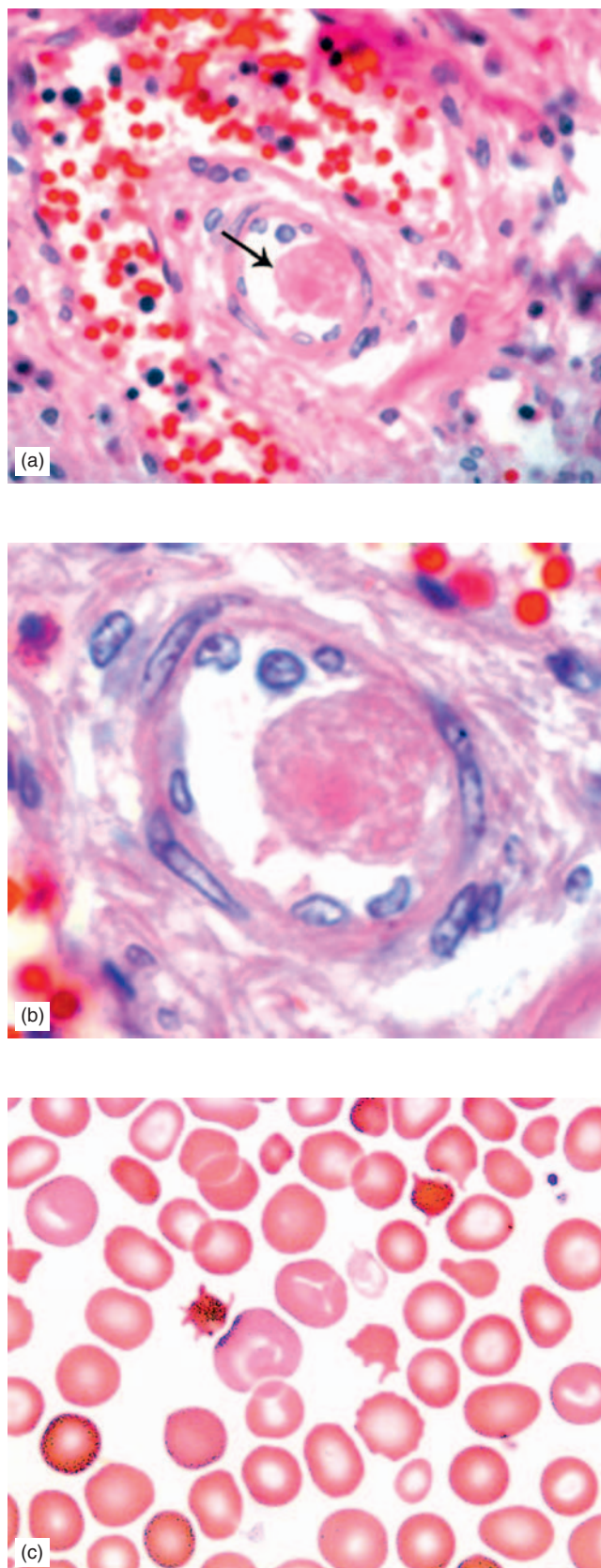


FIGURE 24.5 Platelet/fibrin thrombus in a patient with TTP: (a) low power and (b) high power views; (c) blood smear showing fragmented red cells.

diathesis that most commonly manifests as mucocutaneous bleeding. The main purpose of this section is to provide important diagnostic information useful for evaluating the cause of bleeding in patients suspected of having platelet function disorders. As for the patient with bleeding due to thrombocytopenia, a thorough history and physical examination are important components of the overall diagnostic evaluation to identify the specific abnormality underlying hemorrhage in patients with dysfunctional platelets [87]. The qualitative disorders of platelet function can be divided and discussed within the context of two broad categories based on whether they are inherited and present at birth or are acquired after birth and manifest at some point later in life. Although acquired defects are by far more common, we first discuss the inherited disorders since they cause specific biochemical abnormalities and typically predominantly impair only one of the major aspects of platelet function including adhesion, activation, secretion, aggregation, and formation of a surface for concentrating and facilitating interactions between proteins of the coagulation system [88, 89]. Because for most acquired disorders the specific biochemical abnormality is poorly understood and more than one aspect of platelet function is usually impaired, these disorders are discussed here in the context of their associated underlying medical condition, which is usually evident [90]. Herein, we provide a brief overview of the most common platelet function disorders.

CONGENITAL PLATELET DISORDERS

Bernard–Soulier Syndrome

The Bernard–Soulier syndrome (BSS), previously known as hemorrhagic thrombocytopathic dystrophy, was first reported in 1948 by Jean Bernard and Jean-Pierre Soulier, who described a child from consanguineous parents that had mucocutaneous bleeding out of proportion to what would be expected based solely on the mildly decreased platelet count [88, 91]. Characterized by a prolonged bleeding time, macrothrombocytes (i.e. giant platelets) and thrombocytopenia, with counts ranging between 20,000 and 100,000 platelets/ μL , the BSS is an inherited autosomal recessive hemorrhagic disorder that is characterized by a deficient and/or dysfunctional platelet membrane glycoprotein (GP) complex known as GPIb-IX-V, which is the main receptor for VWF. Although BSS is extremely rare, with only about 100 cases reported in the literature thus far [91], the discovery of this syndrome, together with the finding – from numerous subsequent studies performed in many laboratories – that platelets from these patients display abnormalities in adhesion due to an inability to interact with VWF, ultimately led to the discovery of ristocetin (and botrocetin) and the development of new *in vitro* assays useful in the diagnosis and management of patients with von Willebrand's disease (VWD) and platelet function disorders.

Etiology and Pathogenesis

GPIb-IX-V plays a major role in primary hemostasis as it represents the main platelet surface receptor for VWF.

Because platelets from BSS patients have a defective and/or deficient GPIb-IX-V complex, they are unable to bind the VWF that is present at a high concentration in the sub-endothelial ECM and therefore cannot adhere adequately to sites of vascular injury. GPIb-IX-V is a macromolecular complex that comprises four distinct membrane-spanning glycoproteins, all belonging to the leucine-rich motif containing protein family [92]. Each of these glycoproteins, referred to as GPIb α , GPIb β , GPIX, and GPV, is synthesized and assembled in mature megakaryocytes, the precursors of mature circulating platelets, to form the functional VWF receptor [92–96]. All four subunits of this receptor are encoded by single copy genes (*GPIBA*, *GPIBB*, *GPIX*, and *GPV*) that are exclusively expressed in the platelet lineage [97–100]. GPIb consists of two disulfide linked subunits, GPIb α (135 kDa) and GPIb β (26 kDa), both of which must undergo normal posttranslational processing for the entire GPIb-IX-V complex to be expressed at wild-type levels on the platelet surface [101, 102]. Although GPIX (20 kDa) and GPV (82 kDa) both bind GPIb noncovalently, only the interaction with GPIX is essential for normal expression (and function) of this VWF receptor. Since the associations between GPIb α , GPIb β , and GPIX are required for efficient transport of the entire GPIb-IX-V complex to the platelet membrane, and the absence of even a single subunit can abolish its surface expression, their biosynthesis must be coordinated and tightly regulated [88]. As described later, this is consistent with the fact that loss-of-function mutations have been identified in the genes encoding *GPIBA*, *GPIBB*, and *GPIX* of patients with the BSS. Because, in contrast, no mutations have, as of yet, been identified in the gene encoding *GPV* in BSS patients, *GPV* is likely to be less tightly associated with the other three proteins comprising the GPIb-IX-V complex [103]. The fact that knock-out mice, which have had a targeted disruption of their *GPV* gene, do not develop a BSS-like disorder is consistent with this notion [104, 105]. Although these mice were found to be hyper-responsive to thrombin activation, they demonstrated that a congenital absence of *GPV* does not prevent the surface expression of the heterotrimeric complex GPIb-IX, which itself is capable of VWF binding.

The GPIb-IX-V complex is the major link between the platelet plasma membrane and the underlying actin cytoskeleton, through an intermediate interaction with filamin. Because these interactions restrict the mobility of the spectrin-based membrane skeleton, the loss of an actin filament attachment to the plasma membrane explains the large size of BSS platelets as well as their fragility, both of which likely contribute to the thrombocytopenia observed in patients with this disorder.

Morphology and Immunophenotype

Platelet counts typically are only mildly decreased (80,000–100,000 platelets/ μL) but can vary substantially and even be normal. Because electronic cell counters frequently underestimate the actual platelet count in patients with macrothrombocytes and therefore yield an artificially low mean platelet volume (MPV), a manual platelet count should be performed in patients suspected of having the BSS or

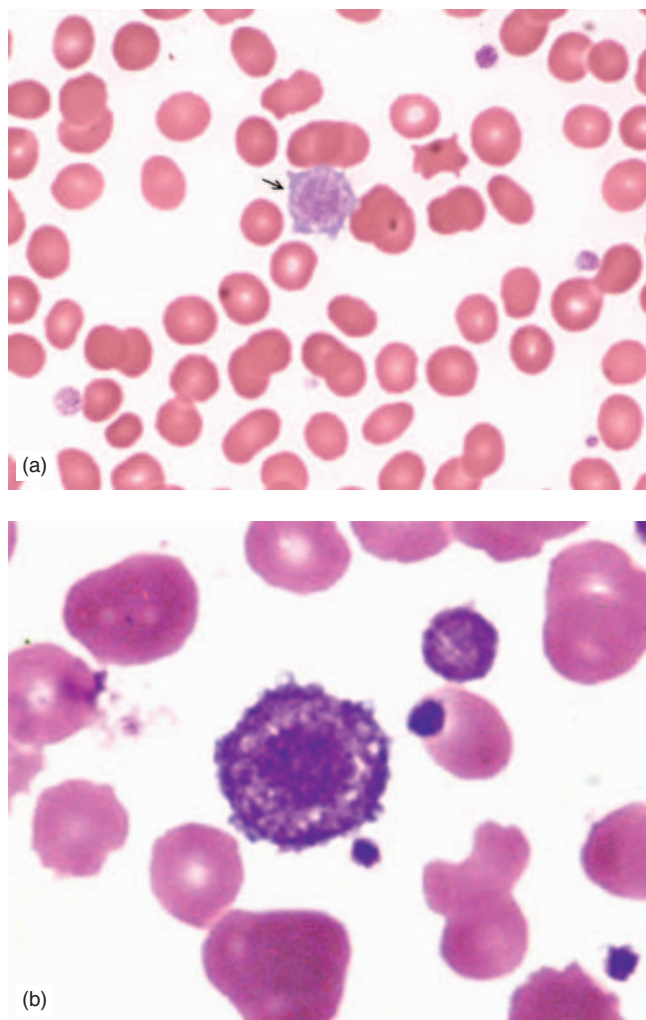


FIGURE 24.6 Blood smear demonstrates giant platelet: (a) low power (arrow) and (b) high power views.

one of the other disorders accompanied by giant platelets (Figure 24.6). During light microscopic examination of peripheral blood smears from patients with the BSS, giant platelets up to 20 μm in diameter are frequently observed and can comprise up to 80% of the entire platelet population. In ultrastructural examinations by electron microscopy, platelets are observed to have rounded shapes (instead of the normal discoid structure) and an abundant supply of granules distributed throughout the cytoplasm except in zones enriched for vacuoles and entangled membrane complexes (Figure 24.7). Precursor structural correlates of these ultrastructural abnormalities have been observed in megakaryocytes, with a dystrophic demarcation membrane system being one of the most common. Ultrastructural abnormalities of the nucleus, such as an increased ploidy in mature megakaryocytes, are also present [106].

Because the BSS is the consequence of an inherited deficiency of the GPIb-IX-V complex, flow cytometric analysis of platelets from suspected patients, using monoclonal antibodies specific for the component proteins GPIb, GPIX, and GPV, represents a rapid diagnostic strategy to

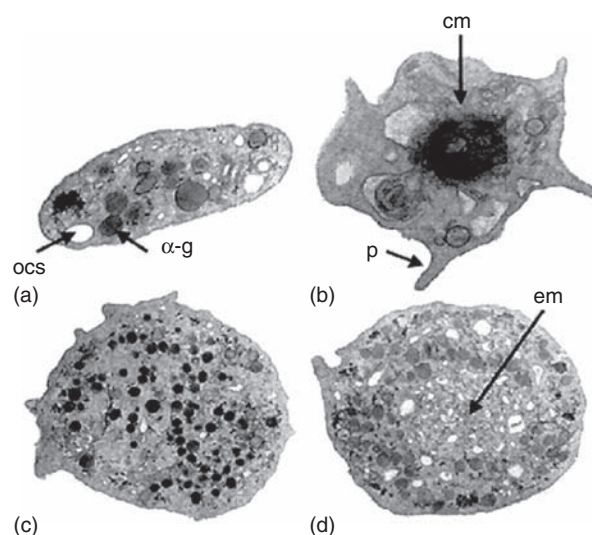


FIGURE 24.7 Electron microscopy of platelets in the BSS. (a) A normal discoid platelet showing both α -granules (α -g) and the open canalicular system (ocs). (b) A normal platelet after thrombin stimulation showing its rounded form, dark central mass (cm) of contractile protein, protruding pseudopods, but no granules. (c) A giant platelet from a BSS patient that is rounded in shape and has abundant granules distributed throughout the cytoplasm. (d) A giant platelet from a May-Hegglin anomaly patient that is similarly appearing except for the cytoplasmic zones rich in entangled membranes (em). From Alan D. Michelson (2007). *Platelets*, 2nd ed. (Figure 57.1), Academic Press, Amsterdam, by permission.

identify individuals who are homozygous and heterozygous for this disorder. With flow cytometry, giant BSS platelets can be analyzed directly in whole blood without having to be separated from the similar-sized red and white blood cells. However, light scatter (LS) gates may require adjustment when evaluating patients with macrothrombocyte syndromes such as the BSS, since forward-LS (F-LS) correlates with platelet size. Because this adjustment can result in some overlap of the F-LS from giant platelets with that from a subpopulation of both red and white blood cells, it is essential to include a platelet-specific monoclonal antibody in the assay as a platelet identifier. The identifier antibody used will depend on the specific platelet disorder that is suspected; for BSS platelets, this antibody cannot be GPIb-, GPIX-, or GPV-specific.

Molecular and Cytogenetic Studies

GPIb α , GPIb β , GPIX, and GPV are encoded by four distinct genes – *GP1BA*, *GP1BB*, *GP9*, and *GP5*, respectively – which are expressed solely within the megakaryocyte/platelet lineage. Almost 45 distinct loss-of-function genetic defects associated with the BSS have been reported; most of these can be reviewed online at the web site for the database of BSS mutations (Figure 24.8) [107, 108]. Because 19 different mutant alleles of *GP1BA*, which is located on chromosome (chr)17p12, have been identified thus far, defects in this gene represent the most common cause of the BSS. This is likely because *GP1BA* encodes GPIb α , which is the largest protein of the complex and bears the VWF-binding site. Although together 25 different mutations have been identified in *GP1BB* ($N = 15$), located on chr22q11.2, and *GP9* ($N = 10$), located on chr3q29, no loss-of-function alleles of

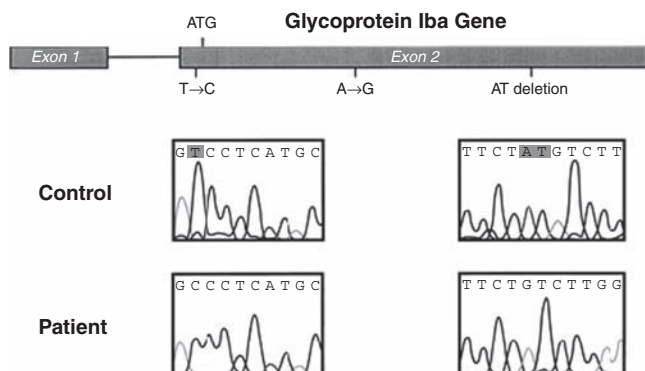


FIGURE 24.8 Mutations found in the GPIIb gene of a BSS patient. Sequencing results from the patient and from a control subject are compared in the bottom panels, with the mutated nucleotides shown enclosed in gray boxes. From Afshar-Kharghan V, López JA. (1997). Bernard–Soulier syndrome caused by a dinucleotide deletion and reading frameshift in the region encoding the glycoprotein Ib alpha transmembrane domain. *Blood* **90**, 2634–43, by permission.

GP5 (chr3q21) have been found in patients with the BSS. As described earlier, this is consistent with the fact that the coordinate expression of GPIIb, GPIIb3, and GPIX, and their subsequent association, is essential for efficient transport of the overall complex to the platelet surface, whereas GPV biosynthesis is known to be expendable for the expression and function of the VWF receptor.

That the BSS usually exhibits an autosomal recessive pattern of inheritance typically means that affected children are born to consanguineous parents, both of which are carriers for (and “passed on”) the same loss-of-function allele of the *GP1BA*, *GP1BB*, or *GP9* gene. There are a number of rare exceptions to this, however, as the disease can occur in association with some syndromes associated with chromosomal abnormalities or can occur in an autosomal dominant manner. This means that rarely the disease can be inherited from just one parent who carries the gene for the abnormality.

Clinical Aspects

The BSS is extremely rare with a prevalence estimated at less than one patient per one million people. Consistent with its autosomal recessive pattern of inheritance, BSS patients can be either male or female. Most of the approximately 100 cases reported since 1948 have presented with an identical disorder to that found in the original patients described by Bernard and Soulier: namely, a severe hemorrhagic defect characterized by a prolonged bleeding time and a low platelet count with very large platelets (i.e. a macrothrombocytopenia). In most of these patients, the bleeding symptoms manifested shortly after birth or during early childhood. Clinical manifestations usually include easy bruisability, petechiae, epistaxis, and gingival bleeding, and less frequently gastrointestinal bleeding and hematuria. In older female patients, menorrhagia is common and can be severe. Episodes of severe bleeding are usually associated with trauma and surgical procedures such as tonsillectomy, appendectomy, and splenectomy, but also occur during dental

extractions and menses. Although the frequency and severity of hemorrhagic episodes can vary from patient to patient, even within the same family, bleeding mainly affects mucocutaneous tissues, with joint bleeds, major hematomas, and intracranial hemorrhages only very rarely being observed.

Quality care for BSS patients involves both prophylactic and therapeutic measures. Effective prophylaxis requires good dental hygiene, educating patients how to locally control nosebleeds, and frequent follow-up for premenopausal women. Although platelet transfusion is the only broadly and consistently effective therapy for severe bleeding, it must be reserved only for those patients with uncontrollable or life-threatening hemorrhage so as to avoid unnecessary exposures to foreign antigens and the possible development of refractoriness to future platelet transfusions due to alloimmunization. However, most BSS patients require transfusions at multiple points in their lives. Alternative therapy with DDAVP has been employed successfully for quite some time. Infusions of recombinant factor VIIa (rFVIIa), also known as NovoSeven®, have been found to be effective in many platelet function disorders and are therefore increasingly being employed. In women of childbearing age, menorrhagia is quite responsive to hormonal regulation therapy in most cases but occasionally requires supplementation with one of the strategies described earlier.

Differential Diagnosis

Functional analysis of platelets by aggregometry, using a patient’s platelet-rich plasma (PRP), is necessary to differentiate the BSS from other rare, inherited platelet function disorders accompanied by macrothrombocytopenia that include (1) *MYH9*-related diseases (i.e. May–Hegglin anomaly, Sebastian syndrome, Fechtner syndrome, and Epstein syndrome); (2) DiGeorge syndrome (i.e. velocardiofacial syndrome [VCFS]); (3) benign Mediterranean macrothrombocytopenia; (4) platelet-type VWD; (5) gray platelet syndrome; (6) Montreal platelet syndrome; (7) macrothrombocytopenia with platelet expression of glycophorin-A; and (8) macrothrombocytopenia with platelet β 1-tubulin Q43P polymorphism. In addition to macrothrombocytopenia, therefore, a diagnosis of the BSS is based on a prolonged bleeding time, defective ristocetin-induced platelet agglutination (RIPA), and low or absent platelet levels of the GPIIb-IX-V complex (CD42a–d) by flow cytometry.

Glanzmann Thrombasthenia

Glanzmann thrombasthenia (GT) was first described in 1918 by Eduard Glanzmann in a patient with recurrent mucocutaneous bleeding, a prolonged bleeding time, and an isolated, rather than clumped, appearance of platelets on a peripheral blood smear. Nearly 40 years later, Braunsteiner and Pakesch described GT as an inherited bleeding disorder characterized by normal size platelets that neither spread on surfaces nor support clot retraction [109]. Caen *et al.* subsequently established the absence of platelet aggregation as the hallmark diagnostic feature of GT in a report on 15 French patients [110]. To date, impaired or absent aggregation to all known physiologic agonists remains the defining characteristic of GT, the most common platelet

function disorder. GT exhibits autosomal recessive inheritance and is caused by a deficiency and/or dysfunction of the α IIb β 3 integrin, a heterodimeric platelet membrane protein also known as GPIIb-IIIa. Platelet-to-platelet interactions essential for thrombus propagation at sites of vascular injury are not possible in GT patients because wild-type α IIb β 3 functions by binding the aggregative plasma proteins fibrinogen and VWF, which in turn converts the initial layer of adherent platelets into a reactive surface that supports continued platelet deposition. Although GT is a rare Mendelian disorder, studies of GT platelets have helped elucidate important structure/function properties of α IIb β 3 and have led to the development of a widely used class of antithrombotic agents including Abciximab, Eptifibatide, and Tirofiban, which function by binding and inhibiting the conformationally active form of this integrin [111].

Etiology and Pathogenesis

GPIIb-IIIa is expressed exclusively by megakaryocytes [26], and under normal conditions, it is the most abundant integral platelet plasma membrane protein, with up to 80,000 copies per cell [112–114]. Such a high surface density is important because α IIb β 3 is a multifaceted receptor that is essential for both initiating and propagating thrombus formation. Despite the fact that GPIb-IX-V mediates the first adhesive interaction of platelets with the subendothelial matrix by binding VWF molecules immobilized within its collagen fibers, these interactions are transient and break if additional contacts between other platelet membrane proteins and the ECM are not made to stabilize them. GPIIb-IIIa is required for successful initiation of thrombus formation as these additional contacts are mediated largely by the activated form of α IIb β 3, which binds tightly to VWF through a high-affinity interaction with the peptide sequence Arg–Gly–Asp (RGD). Activated α IIb β 3 is essential for thrombus growth as well, because it is also the principal receptor for plasma fibrinogen and VWF, the soluble proteins required for continued deposition of platelets through aggregation, the *in vivo* process by which nonadherent platelets are tethered to adherent platelets in the initial monolayer. Wild-type α IIb β 3 molecules are also essential for platelets, suspended in plasma, to aggregate in response to the various agonists that are used *in vitro* to test platelet functioning by aggregometry. Central for normal α IIb β 3 function is its ability to undergo an inducible allosteric switch from a bent, low-affinity resting form to an extended, active conformation that has a high affinity for the adhesive and aggregative ligands mentioned earlier. This affinity modulation is initiated when various platelet adhesion and/or G-protein-coupled receptors are stimulated, through interactions with one or more of several agonists, to generate second messengers that bind the cytoplasmic tails of α IIb β 3 and, in a process referred to as “inside-out” signaling, [115–118] trigger a switch of its extracellular domain to a conformation competent for binding multivalent VWF and divalent fibrinogen molecules. Binding of α IIb β 3 to either molecule induces “outside-in” signaling, a process that leads to the assembly of signaling and actin/myosin complexes on the cytoplasmic domains of activated integrins [119].

Because α IIb β 3 is deficient and/or defective in GT, platelets from these patients are unable to stably adhere to or aggregate at sites of vascular injury. Additionally, aggregation is absent

or markedly diminished in response to all agonists used in aggregometry except ristocetin, since this bacterial glycopeptide induces agglutination (not aggregation) through an integrin-independent mechanism that promotes interaction between VWF and GPIb-IX-V, both of which are wild-type in GT. The inability of GT platelets to stably adhere to or aggregate on exposed subendothelial tissues is consistent with the fact that they do not spread on these surfaces or support clot retraction.

Morphology and Immunophenotype

Platelet counts in patients with GT are typically normal, whether performed with electronic cell counters or manually. Routinely measured parameters of platelet structure, such as MPV, are also generally within normal limits. In light microscopic examination of peripheral blood smears and ultrastructural examinations by electron microscopy, platelets from GT patients are morphologically normal. Because GT is caused by a deficiency of GPIIb-IIIa, also designated CD41/CD61, flow cytometric analysis of platelets from suspected patients, using monoclonal antibodies specific for the component proteins α IIb and β 3, represents a rapid diagnostic strategy to identify homozygotes and heterozygotes for this disorder [120, 121]. Once platelet function studies demonstrate absent aggregation to all agonists, flow cytometry is the method of choice to analyze platelet surface glycoproteins and screen for GT because it can be performed on small blood samples and allows diagnoses to be made in children.

Molecular and Cytogenetic Features

Like all integrins, α IIb β 3 comprises an alpha (α IIb) and beta (β 3) heterodimer with the two subunits associated noncovalently in a 1:1 complex. Since association between α IIb and β 3 – which are encoded by the single copy genes *ITGA2B* and *ITGB3*, respectively, located adjacently on chr17q21-23 – is required for efficient transport of this integrin to the platelet membrane, absence of either subunit can abolish its surface expression. This is consistent with the fact that (1) non-complexed or incorrectly folded subunits are not processed in the Golgi apparatus and undergo intracellular degradation and (2) loss-of-function mutations have been identified in both *ITGA2B* and *ITGB3* in unrelated GT patients. A database is available on the Internet that currently lists more than 100 different GT-causing mutations. Although large deletions are rare, single-base-substitution abnormalities encoding missense, nonsense, and splice-site mutations are common, as are small deletions and insertions. Missense mutants of both *ITGA2B* and *ITGB3*, which encode aberrant α IIb and β 3 proteins, respectively, that differ from the wild-type subunits by a single amino acid, represent the most common overall cause of GT.

Consistent with its autosomal recessive inheritance, GT patients are most frequently born to consanguineous parents and are homozygous for a mutation in either *ITGA2B* or *ITGB3*. However, a large number of compound heterozygous patients with two distinct loss-of-function *ITGA2B* or *ITGB3* alleles have been reported. *ITGA2B* mutations, which have been found in all functional regions of this 30 exon containing approximately 17 kb gene, are somewhat more common than mutations in the approximately

65kb *ITGB3* locus, which have been identified in most of its 15 exons and flanking intronic sequences. Although most patients have *ITGA2B* or *ITGB3* defects that result in either the absence of (type I) or a severely decreased level of (type II) α Ib β 3 on the surface of their platelets, some carry one (or two) of a number of missense *ITGA2B* or *ITGB3* alleles that encode a dysfunctional form of the protein. Such patients comprise a category of this disorder referred to as variant GT because they express immunologically recognizable α Ib β 3 molecules that are unable to bind fibrinogen. Studies performed on subjects with variant GT, which differ from those with type I or type II disease and comprise a heterogeneous collection of patients with a broad range of bleeding diatheses from severe to mild, have been very useful in elucidating integrin function.

Although α Ib β 3 is normally found solely in platelets and because α Ib is expressed only by megakaryocytes, β 3 is expressed by a number of other cell types including endothelial cells, osteoblasts, chondrocytes, fibroblasts, smooth muscle cells, monocytes, and certain lymphocytes. Despite this and the fact that platelets express a second, less abundant (i.e. \sim 50 copies/cell) β 3-integrin, the vitronectin receptor α V β 3, patients with *ITGB3* defects do not appear to have a more severe form of the disease for unknown reasons.

Clinical Aspects

As an autosomal recessive disorder, GT affects both males and females. Patients typically manifest moderate to severe mucocutaneous bleeding, with purpura, epistaxis, gingival hemorrhage, and menorrhagia being the most common at presentation [122, 143]. However, there is considerable variability in the associated bleeding diatheses, even in patients with the same gene abnormality, with some manifesting only easy bruisability and others severe, life-threatening hemorrhages. Although genitourinary and gastrointestinal hemorrhages also occur, deep-visceral, intra-articular, and intramuscular bleeding is rare. Although all patients share the hallmark diagnostic feature of GT, that is, the absence of platelet aggregation in response to all known agonists, they differ with respect to whether their platelets support residual clot retraction or express immunologically detectable surface α Ib β 3 molecules. Patients whose platelets lack clot retraction and express $<5\%$ of the mean normal α Ib β 3 level on their surface are defined as having type I GT. Platelets from patients with type II disease demonstrate residual clot retraction and express up to 20% of the normal α Ib β 3 level. In contrast, platelets from variant GT patients have nearly normal to normal surface levels of a dysfunctional α Ib β 3 molecule. Consequently, although platelets from all patients with variant disease lack agonist-induced aggregation, they have a variable ability to support clot retraction ranging from normal to absent, which likely underlies their heterogeneity in bleeding symptoms that range from mild to severe.

Platelet transfusion is the treatment of choice for severe bleeding episodes, but some patients become refractory due to alloimmunization, presumably because they lack preexisting tolerance to the wild-type allele of either the α Ib or the β 3 subunit of GPIIb-IIIa, the mismatched antigen(s) of the HLA system, or both. Although rFVIIa infusion may represent a therapeutic alternative to platelet transfusion, especially

in those with antiplatelet antibodies, it evidently is not effective in all patients. However, invasive procedures or surgery can be performed in rFVIIa-unresponsive alloimmunized patients if their platelet antibody titers are first transiently reduced using protein-A immunoabsorption. As a last resort, bone marrow transplantation can be used successfully to treat and cure severe hemorrhagic, alloimmunized GT patients.

Differential Diagnosis

GT is the only disease in which platelet aggregation is absent or markedly impaired in response to all physiologic agonists used in aggregometry, including adenosine diphosphate (ADP), collagen, epinephrine, arachidonic acid, and thrombin. Because platelets from GT patients are also normal in size and agglutinate normally in the RIPA assay, one can readily exclude the BSS, as platelets from these patients are unusually large and despite aggregating normally in response to all agonists do not agglutinate in the RIPA assay. Furthermore, additional inherited disorders can be excluded including the nonmuscle myosin heavy chain IIA-associated syndromes (i.e., May-Hegglin anomaly, Fechtner syndrome, Sebastian syndrome, and Epstein syndrome) and the Montreal platelet syndrome, which are all associated with abnormally large platelets, and the Wiskott-Aldrich syndrome (WAS), in which abnormally small platelets are observed.

Although congenital GT patients typically present with hemorrhage in early childhood, acquired causes must be ruled out when there is no family history. Acquired GT has been reported in patients with acute promyelocytic leukemia (APL) [123]. Analogous to congenital GT, platelets from these patients were deficient in α Ib β 3 and failed to aggregate in response to all agonists. Although not definitively demonstrated at the DNA level, a disruption of either *ITGA2B* or *ITGB3* is the likely etiology of acquired GT in this setting as chr17 breakpoints underlying the chr15-17 translocations in most APL patients are heterogeneous and known to occur occasionally at 17q21, the location of these genes [124]. Other possible etiologies in the differential diagnosis for congenital bleeding disorders can be excluded on this basis. For instance, impaired platelet aggregation, specifically in response to ADP or collagen, implies defects in either the primary receptors for these agonists or the signaling pathways they induce.

Wiskott-Aldrich Syndrome

Wiskott-Aldrich syndrome (WAS) is an X-linked congenital disorder characterized by severe thrombocytopenia, immunodeficiency, recurrent infections, and eczema [125–129]. The Wiskott-Aldrich syndrome protein (WASP) structural gene (*WASP*) resides at Xp11.22-23. About 300 *WASP* mutations have been discovered, including single-base substitutions, deletions, insertions, and splice-site mutations [128, 130–133]. The WASP appears to play critical roles in signal transduction and in regulating cytoskeletal reorganization [130, 131].

Approximately 40% of the patients with WAS demonstrate an associated autoimmune disorder. Hemolytic

anemia, vasculitis, nephritis, and inflammatory bowel disorders are the most frequently encountered [128, 134]. Thrombocytopenia is usually severe and $<50,000/\mu\text{L}$. Platelets are small (about half the average size of normal platelets) and are associated with functional defects and shortened survival [129, 135]. A variant of WAS with milder clinical presentation exists, which is referred to as X-linked thrombocytopenia (XLT) [130].

Disorders of Platelet Secretion and Granule Deficiencies

These disorders fall into two major categories: (1) deficiencies in the platelet granules or their contents and (2) defects in the ability of platelets to release their contents [136, 137]. Platelet dense granules contain serotonin, pyrophosphate, calcium, ADP, and ATP [138, 139]. Platelet α -granules contain numerous proteins such as fibrinogen, platelet factor 4, platelet-derived growth factor, β -thromboglobulin, factor V, VWF, and high-molecular-weight kininogen [137, 140]. In addition to dense and α -granules, platelets contain vesicles with acid hydrolases. These vesicles are involved in the arachidonic acid pathways and thromboxane A_2 production.

Dense Granule Deficiency

Dense granule deficiency (or δ -storage pool disease) is characterized by marked decrease or absence of platelet dense granules [136–138, 141]. Platelets are normal in size and show unremarkable ultrastructural features, except for a marked decline or absence of dense granules [136, 138, 141].

Patients demonstrate a mild to moderate bleeding diathesis such as easy bruising, epistaxis, gingival bleeding, and menorrhagia. The bleeding time is prolonged and *in vitro* platelet function studies reveal absence of the second wave of aggregation with either ADP or epinephrine and an impaired aggregation response to collagen [142]. Dense granule deficiency has been associated with a number of other congenital abnormalities such as Hermansky–Pudlak syndrome (oculocutaneous albinism and increased ceroid in the monocytic/histiocytic system), Chediak–Higashi syndrome, WAS, and congenital thrombocytopenia with absent radii (TAR) syndrome [125, 143, 144].

Alpha-Granule Deficiency

Platelet α -granule deficiency, *gray platelet syndrome* or *α -storage pool disease*, is caused by the reduction or absence of platelet α -granules (Figure 24.9) [136, 145–147]. The affected patients have a history of a bleeding diathesis and demonstrate a mild thrombocytopenia. Platelets appear grayish in blood smears stained with Wright's stain. Ultrastructural studies reveal absence or markedly reduced numbers of α -granules in the affected platelets. The affected megakaryocytes also show absent or decreased numbers of α -granules [148]. The basic defect seems to be the inability

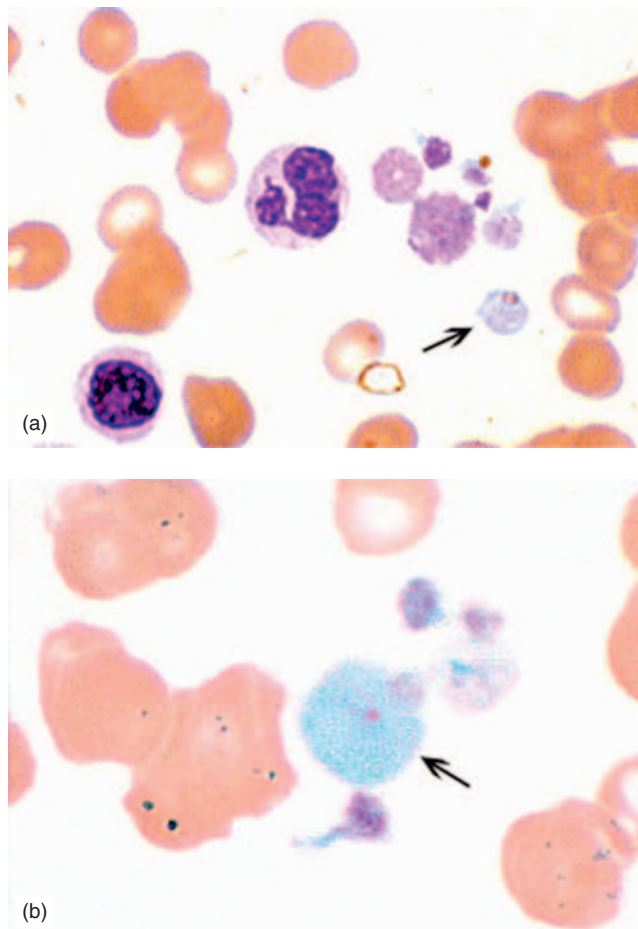


FIGURE 24.9 Blood smears demonstrate markedly hypogranular platelets (a and b, arrows).

of megakaryocytes to transfer endogenously synthesized proteins into α -granule precursors [145]. Affected patients show a prolonged bleeding time and variable responses in platelet aggregation studies. The most consistent finding is impairment of thrombin-induced aggregation [142].

Abnormalities in Platelet Arachidonic Acid Pathways

Abnormalities of arachidonic acid pathways are extremely rare and are of two major types: (1) defect in the release of arachidonic acid from phospholipids and (2) deficiencies of cyclooxygenase or thromboxane synthetase [142, 149]. Affected patients are usually adults and often demonstrate mild to moderate hemorrhages [142]. Severe bleeding is rare [150].

Formation of thromboxane A_2 is one of the major responses of platelets during activation. Thromboxane A_2 is necessary for platelet secretion during the stimulation of platelets with ADP, epinephrine, and low concentration of collagen and thrombin. Thromboxane A_2 is also a potent vasoconstrictor [142].

ACQUIRED PLATELET DISORDERS

Drug-Induced Disorders

Many commonly used drugs are known to affect platelet function. Although some of these agents were developed mainly for treating patients at risk for thromboembolism because of their ability to inhibit specifically one (or more) of the several distinct molecular events required for normal platelet function, and therefore to impair primary hemostasis, most were developed for clinical indications unrelated to their hemostatic effects and were found subsequently to nonspecifically inhibit platelet function. Because the mechanism(s) by which the agents in the former group impair platelet function have been studied extensively and are well established, they will only be discussed briefly here. However, because much of our knowledge in this area on drugs from the latter, much larger group comes from *in vitro* studies performed on platelets exposed to one pharmacologic agent at a time, the overall impact on the hemostasis system *in vivo* has not been established for most of them. Furthermore, the clinical relevance of this knowledge is not clear since most patients are administered more than one drug simultaneously. Inhibitors of platelet cyclooxygenase-1 (COX-1), including aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs), are among the most commonly used medications.

Aspirin

Aspirin irreversibly inactivates cyclooxygenase and thereby inhibits production of thromboxane A_2 from arachidonic acid and impairs platelet secretion [151]. The end result is defective platelet aggregation and prolonged bleeding time. Prolongation of the bleeding time may last up to 4 days after administration of aspirin is stopped [152, 153]. Ethanol ingestion may enhance prolongation of the bleeding time in patients who take aspirin [154]. Other NSAIDs also inhibit COX-1 but do so reversibly.

Beta-Lactam Antibiotics

Beta-lactam antibiotics, such as penicillin and cephalosporin derivatives, may prolong bleeding time and induce abnormal platelet aggregation [155–157]. These antibiotics seem to interfere with the function of platelet membrane integrins such as GPIIb-IIIa and GPIa-IIa. The effect is dose- and duration-dependent.

Others

Excessive garlic ingestion may induce platelet dysfunction and inhibits cyclooxygenase activity [158, 159]. Long-term dietary supplementation with marine oils reduces the platelet content of arachidonic acid and may cause abnormal platelet aggregation and slight prolongation of the bleeding time [160]. Dextran may slightly prolong the bleeding time without increasing operative or postoperative bleeding. Therefore, it has been used for the prevention of postsurgical thromboembolic complications [161, 162].

PLATELET DYSFUNCTION ASSOCIATED WITH PATHOLOGIC CONDITIONS

Cardiopulmonary Bypass

Prolonged bleeding time, abnormal platelet aggregation, and thrombocytopenia are some of the common features of cardiopulmonary bypass [163, 164]. During bypass surgery, platelets adhere to fibrinogen absorbed by the bypass circuit. Bypass procedures also enhance thrombin and ADP generation and complement activation [165]. Mechanical trauma from the bypass pump may also degranulate platelets.

Chronic Renal Failure

Uremia may lead to platelet dysfunction and abnormal aggregation [166, 167]. The bleeding time is often prolonged, and there may be bleeding manifestations such as purpura, epistaxis, menorrhagia, gastrointestinal bleeding, and hematuria.

Hematologic Disorders

Abnormal platelet function and morphology may occur in association with myelodysplastic syndromes, myeloproliferative disorders, and acute myelogenous leukemia (see Chapters 9–12). Abnormal platelet functions include decreased platelet aggregation and secretion in response to ADP, epinephrine, and collagen, and reduced platelet procoagulant activity. Morphologic changes include abnormal shapes, giant forms, and hypogranularity. A case of hairy-cell leukemia with abnormal platelet morphology and severe platelet dysfunction has been reported [168].

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